RISK STRATIFICATION IN PRIMARY BILIARY CIRRHOSIS

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Cover designed by Joke Blijleven, kunstschilderes
Lay-out & printing: Gildeprint, Enschede, the Netherlands

The work presented in this thesis was conducted at the Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center Rotterdam, the Netherlands.

ISBN 978-94-6233-130-3

Financial support for printing of this thesis was provided by: Zambon Nederland, de Nederlandse Vereniging voor Hepatologie en afdeling maag-, darm- en leverziekten van het Erasmus MC, Rotterdam.

RISK STRATIFICATION IN PRIMARY BILIARY CIRRHOSIS

Risicostratificatie in primaire biliaire cirrose

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 25 november 2015 om 13.30 uur

door

Willem Johannes Lammers
geboren te Ede

Erasmus University Rotterdam

PROMOTIECOMMISSIE

Promotor:	Prof.dr. H.L.A. Janssen
Overige leden:	Prof.dr. U.H.W. Beuers Prof.dr. H.J. Metselaar Prof.dr. E.W. Steyerberg
Copromotoren:	Dr. H.R. van Buuren Dr. B.E. Hansen

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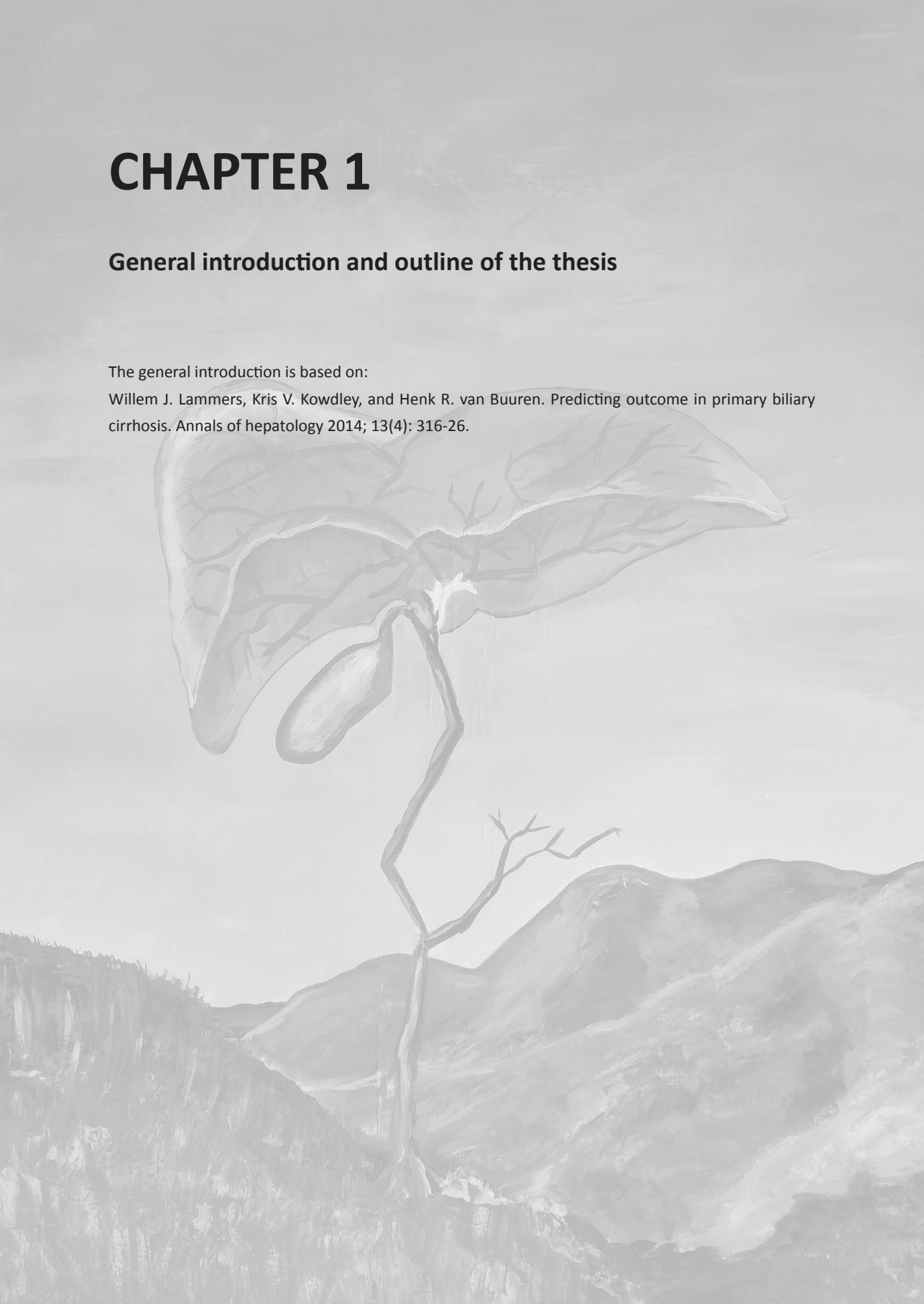
CHAPTER 1

General introduction and outline of the thesis

The general introduction is based on:
Willem J. Lammers, Kris V. Kowdley, and Henk R. van Buuren. Predicting outcome in primary biliary cirrhosis. Annals of hepatology 2014; 13(4): 316-26.



Primary biliary cirrhosis (PBC) is an usually slowly progressive autoimmune liver disease characterized by a chronic non-suppurative destructive cholangitis.[1] Ultimately the disease may lead to severe bile duct loss (ductopenia) accompanied by increasing fibrosis, finally resulting in cirrhosis, liver failure and premature death. Liver transplantation has a significant beneficial effect on outcome in patients who progress to end-stage disease, however this ultimate therapeutic option is only available for a selected group of patients. Ursodeoxycholic acid (UDCA) is the only established medical treatment over the past 25 years. It is generally accepted that UDCA improves prognosis in the majority of cases. However, the response to treatment is insufficient in a subset of patients who still have an unmet medical need for effective additional therapy. Timely and reliable assessment of the response to therapy and prediction of outcome is of key importance in clinical management and an essential requirement for patients counselling and timing of diagnostic and therapeutic interventions.

WHAT'S IN A NAME?

The name primary biliary cirrhosis was first mentioned in 1950,[2] although earlier reports described syndromes similar to PBC.[3] Contrary to the name, generally considered a classical misnomer,[1] cirrhosis is only manifest in the late stages of the disease. While the disease was often diagnosed in a late, cirrhotic stage in the past, the majority of patients are nowadays diagnosed with earlier stage disease and a substantial number will likely not develop cirrhosis. Currently there is strong support among patient support groups and the professional hepatology community alike for the initiative, to change the name. In particular, to keep the acronym PBC, the new name "primary biliary cholangitis" has been proposed.[4]

EPIDEMIOLOGY

PBC is a relatively rare liver disease typically affecting middle-aged females, with a male-female ratio of 1:9.[5] Reported incidence rates are varying from 0.33 to 5.8 per 100,000 persons/year and prevalence rates from 1.91 to 40.2 per 100,000 persons.[6] The incidence in the Netherlands is relatively low with a rate of 1.1 per 100.000 persons/year, however increasing incidence and prevalence rates have been observed.[7] The differences in reported prevalence rates are partly explained by differences in the way cases were selected and in awareness of the disease among countries. On the other hand, genetic differences between populations, as well as differences in environmental circumstances might explain differences in prevalence.

PATHOGENESIS

PBC is considered to be an autoimmune liver disease. The female predominance and the frequently coexistence of other autoimmune conditions, such as Sjögren syndrome, thyreoiditis, Raynaud's syndrome, coeliac disease, sclerodermia/CREST syndrome and rheumatoid arthritis, and last but not least the presence of autoantibodies are strong indicators of an autoimmune origin.[8, 9]

The pathogenesis of PBC is still unresolved, but there is strong evidence that PBC is an condition that develops from a complex interaction of genetic predisposition and environmental triggers,[10-12] leading to immunoregulatory changes and finally resulting in destruction of intrahepatic bile ducts and progressive fibrosis.[13]

The impact of genetics on the development of PBC has been suggested by numerous studies. A remarkable study in sixteen monozygote twins, thus genetically identical persons, showed that in 5 of 8 monozygotic twin sets both individuals had PBC while among the dizygotic twins no concordance for PBC was found.[14] It has also been shown that first-degree family members of PBC patients, in particular female relatives, have a higher risk of getting PBC.[9, 15, 16] Further evidence has been provided by genome-wide-association studies (GWAS) that elucidated genetic risk loci for PBC.[17, 18]

Environmental risk factors for PBC development have been described by well-defined observational studies, such as a positive family history, recurrent urinary tract infections, (past) cigarette smoking, use of reproductive hormone replacement, and frequent use of nail polish or hair dye.[9, 15, 19, 20]

How the exposure to environmental factors in a genetically susceptible individual may trigger the loss of tolerance against autoantigens is still incompletely understood. Most autoantibodies found in PBC are reacting against antigens on the inner cell membrane of mitochondria that contain lipoic acid (antimitochondrial autoantibodies, AMA),[21] especially to the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2). Some studies suggest that external factors (e.g. bacteria) have epitopes comparable to PDC-E2 and subsequently can trigger a cross-reaction of the immune system (molecular mimicry hypothesis).[22, 23] Others suggest that xenobiotics alter host antigens or form complexes leading to loss of tolerance of the immune system.[24]

Interestingly, the autoimmune reaction is specifically directed against the mitochondria of intrahepatic cholangiocytes, despite the presence of mitochondrial antigens in all nucleated body cells. Recent findings suggest an unique immunobiology of cholangiocytes; after apoptosis of cholangiocytes PDC-E2 stays intact and immunologically active in contrast to PDC-E2 in other cell types.[25, 26] Subsequently, a specific auto immune response is triggered, that may play a role in the destructive autoimmune process.[21, 27] Clearly further research is needed to unravel the mysterious pathophysiology of PBC and hopefully this will provide clues how to prevent the disease and to treat it effectively in an early stage.

CLINICAL PRESENTATION

Nowadays, about 60% of patients do not have any symptoms at time of diagnosis, although most asymptomatic patients will develop symptoms over time.[28] The number of asymptomatic patients has been increasing over the past decades.[29] Fatigue and pruritus are the most prevalent symptoms in PBC and can have a major negative effect on perceived quality of life.[30] Fatigue has the highest impact, especially in patients with a poor social network.[31] Unfortunately, limited progress has been made in unravelling the etiopathogenesis of this symptom and no effective treatment is available. Pruritus is another common symptom with a potential major effect on mood and well-being. The severity of pruritus may vary periodically and is unrelated with disease stage and severity. Pruritus is usually most severe during the evening and night. Several therapeutic options are available but the results are frequently unsatisfactory.[32]

Other less frequent symptoms are sicca syndrome, occasional pain in the right upper abdominal quadrant, musculoskeletal pain, arthralgia and xanthomas. A minority of patients present initially with signs of decompensated liver disease, such as jaundice, ascites, bleeding from oesophageal varices or hepatic encephalopathy.

DIAGNOSIS

The diagnosis of PBC is usually straightforward and is based on biochemical, serological and/or histological features. In patients with or without symptoms with otherwise unexplained elevated alkaline phosphatase levels, for at least six months in the presence of AMA or AMA type M2 antibodies, PBC can be diagnosed with confidence.[32, 33] Liver biopsy is no longer considered essential for diagnosis but can be helpful to assess the stage and activity of the disease. Liver biopsy can also be indicated when the diagnosis is considered in the absence of specific antibodies and in case of suspected PBC-autoimmune hepatitis overlap syndrome.[32]

Up to 95% of PBC patients have AMA autoantibodies against PDC-E2 antibodies detectable in serum.[34] Additionally, about half of PBC patients have antinuclear autoantibodies (ANA). Most specific for PBC are ANA reacting against the nuclear envelop, such as gp210 and antigens that are recognized as multiple nuclear dots with immunofluorescence microscopy, such as sp100. Interestingly, up to 85% of AMA-negative patients are ANA-positive.[21] Therefore these antibodies may be useful to establish the diagnosis PBC in AMA-negative patients.[35]

TREATMENT

Treatment options in PBC are limited. Liver transplantation is an important treatment modality with excellent results,[36] but is an option only for patients with end-stage liver disease. In addition, PBC can recur in the transplanted liver although the course of recurrent PBC is usually mild. Currently, UDCA is the only approved medical treatment and advised in doses of 13-15mg/kg/day as first-line treatment in PBC.[32, 33] UDCA is a hydrophilic bile acid that can protect hepatocytes and cholangiocytes from the toxic effects of hydrophobic bile acids. An important proposed mode of action is that UDCA stimulates hepatocellular secretion of hydrophobic bile acids and other potential hepatoxins.[37]

UDCA has been shown to improve liver biochemistry, liver histology and transplant-free survival of PBC patients. A beneficial effect on symptoms has not been documented. Unfortunately, about 40% of patients have an insufficient response to UDCA and these patients should be considered for additional, second-line therapies.[38-42]

Considering the presumed autoimmune etiology of PBC, the efficacy of a large number of immunosuppressive and immunomodulating agents, such as prednisone, D-penicillamine, azathioprine, methotrexate, chorambucil, malotilate and rituximab, has been evaluated during the last decades. These studies were largely negative or showed important adverse treatment effects.[32, 33] More promising results have been reported with budesonide, a glucocorticoid with an extensive first-pass metabolism in the liver. The potential adverse effect profile of budesonide may be superior to that of prednisone and this drug may be an option in non-cirrhotic PBC.[43-45] The potential long-term adverse effects of corticosteroids remain of concern, such as the negative effect on bone mass.[46, 47] The results of a recent controlled trial are awaited with much interest.

A potential viral etiology of PBC has been presumed[48] and antiviral therapies have been assessed,[49, 50] though further research is required.

Currently, a number of new agents are under evaluation that act as agonists of nuclear receptors, such as fibrates and obeticholic acid (OCA, INT-747). The evidence of beneficial effects of fibrates in addition to UDCA is emerging,[51-56] although further evidence from phase 3 randomized placebo-controlled trials is awaited. Fibrates are acting on peroxisome proliferator-activated receptors (PPAR), which are important in lipid and energy homeostasis.[57] It is suggested that activation of PPAR can down regulate bile acid uptake and synthesis in hepatocytes,[54] however the mechanisms leading to anticholestatic effects of fibrates are not completely understood. Recently promising results have been obtained with OCA, a derivative of the bile acid chenodeoxycholic acid (CDCA).[58, 59] In contrast to UDCA, OCA specifically acts on the farnesoid X receptor (FXR), which plays an important role in bile acid synthesis, transport and metabolism. It seems realistic that within a few years evidence-based second-line treatment for PBC will be a reality. The selection of optimal candidate patients for these new therapies is a major challenge.

PROGNOSIS

The natural course of PBC is slowly progressive spanning 10-40 years. The life expectancy of affected patients is worse compared with the general population, but on an individual basis the course of the disease and the prognosis vary greatly. Currently, patients are more likely to be asymptomatic and diagnosed at earlier stages of the disease.[28, 29, 60] **Table 1** summarizes studies published in the last fifteen years reporting 5- and 10- year liver transplant-free survival rates based on Kaplan Meier estimates. The reported differences in outcome are probably attributable to differences in study populations and variability with respect to duration and dose of treatment with UDCA.

Impact of UDCA treatment on the natural course of PBC

With the introduction of UDCA the prognosis of PBC has markedly improved. Besides an improvement of biochemistry several studies with extended follow-up data of earlier published randomized, placebo-controlled UDCA trials showed that UDCA can delay histological progression after several years, but does not regress fibrosis.[61-63]

Its efficacy in terms of survival benefit has been subject of debate over the past decades. Several meta-analyses have failed to show a beneficial effect of UDCA in PBC.[64-66] However, only a few of the included studies in these meta-analyses lasted longer than 24 months, a very short period to demonstrate effects on transplant-free survival, and most studies were clearly underpowered. In contrast, a pooled analysis of individual patient data from the 3 largest placebo-controlled double-blind trials with extended follow-up data from one center, showed an improvement in survival with UDCA after four years of treatment.[67] One meta-analysis showed that the use of UDCA in studies that incorporated placebo control, long-term follow-up (more than 2 years) or larger numbers of patients (more than 100 patients) were associated with both improved serum liver biochemical tests and reduced incidence of liver transplantation or death.[68] Some other studies have shown that UDCA-treated patients with early stage disease have survival rates comparable with an age- and sex-matched controlled population in contrast to UDCA-treated patients with advanced disease.[69, 70] In summary, there is strong evidence to support the use of UDCA to delay the progression of PBC and currently it remains the only licensed medical therapy.

Table 1. Reported 5-year and 10-year transplant-free survival in primary biliary cirrhosis

Group[ref]	Year	N	UDCA treatment Yes/no	Cohort characterization	5-year transplant-free survival	10-year transplant-free survival
Van Hoogstraten et al.[71]	1999	203	Yes	Multicenter	79%	NA
Poupon et al.[72]	1999	225	Yes	Multicenter	80% (*7-year*)	78%*
Papatheodoridis et al. [73]	2002	86	Yes and no	Single center	80%	38%
Chan et al.[74]	2005	69	Yes	Single center	77%	NA
		140	No		78%	NA
Corpechot et al.[69]	2005	262	Yes	Multicenter	84%	66%
Ter Borg et al.[70]	2006	279	Yes	Multicenter	87%	71%
Parés et al.[38]	2006	192	Yes	Single center	93% (*7-year*)	NA
Corpechot et al.[39]	2008	292	Yes	Single centre	94%	85%*
Myers et al.[75]	2009	137	Yes and no	Population-based administrative data	80%	68%
Kuiper et al.[40]	2009	375	Yes	Multicenter	90%	78%
Floreani et al.[29]	2011	327	Yes	Single center	NA	79%*
Zhao et al.[76]	2011	147	NA	Single center Chinese population	79%	NA
Zhang et al.[77]	2013	187	Yes	Single center	86%	63%
Papastergiou et al.[78]	2013	86	Yes	Single center	94%	94%
		129	No		92%	80%

*cumulative probability of survival

Prognostic factors

Histological stage

Severity of disease in PBC is based on the Scheuer[14] and Ludwig[1] histologic scoring systems, both recognizing 4 stages. Early histological stages are associated with favourable prognosis. The last phase, or cirrhotic phase, is irreversible and classically only this stage is associated with an increased risk of liver decompensation and development of hepatocellular carcinoma (HCC).[33, 79] Thus, liver histology is a strong prognostic factor.

A particular variant form of PBC, the premature ductopenic variant, is characterized by rapid, excessive bile duct loss in relation to the amount of fibrosis. In individuals with this subtype, severe cholestasis with progressive jaundice and marked hypercholesterolemia may require liver transplantation well before the development of cirrhosis.[80]

The histological progression of PBC was assessed in patients originally included in a clinical trial of D-penicillamine.[81] Since this agent does not delay histological progression,[82] this study is considered as representative of histological progression in treatment-naïve PBC patients. Approximately 80% of patients had histological progression of at least one stage during a median follow-up of 3 years, and 31% with stage I disease progressed to cirrhosis within 4 years. Another study followed-up 183 patients treated with UDCA and reported a 4% incidence of cirrhosis at 5 years in patients with stage I disease,[83] suggesting that UDCA delays histological progression.

Several other histologic features have been described as important prognostic parameters of worse outcome in PBC, such as central and periportal cholestasis,[79, 84] periportal cell necrosis and piecemeal necrosis,[83, 84] interface hepatitis,[83] and ductopenia.[62] Many of these histological features are not systematically included in the Ludwig and Scheuer histological scoring systems; in fact, an expert panel on PBC, working under the auspices of the American Association for the Study of Liver Disease (AASLD), agreed that histology should neither be included in prognostic scoring models nor used as a primary endpoint in clinical trials.[85] A recently proposed histologic scoring system taking into account several of the histological features discussed awaits further validation.[86]

Serological factors

AMA are highly specific for PBC and a cornerstone for establishment of the diagnosis. Patients having positive AMA in combination with normal serum liver biochemical tests and without symptoms are likely to develop PBC over time.[87] However, neither AMA status nor AMA titer has been shown to be correlated with prognosis.[88, 89] AMA subtypes were found to be associated with a progressive course in some studies,[90] but this was not confirmed by others.[91, 92]

It has been suggested that patients with ANA of the anti-gp210 subtype have more active disease and are more likely to develop liver failure.[93-95]

Biochemical prognostic factors

From a diagnostic point of view increased serum alkaline phosphatase with or without increased gamma-glutamyl transpeptidase (γ-GT) are of key importance, and both are considered as early markers of cholestasis in contrast to elevated serum total bilirubin values, which are clearly suggestive of more advanced disease.[96]

It has been known for several decades that serum bilirubin is one of the most powerful predictors of prognosis in PBC and this variable has been incorporated in most scoring and prediction models. A classical study demonstrated a two-phase pattern of bilirubin during the course of the disease;[97] a first phase in which serum bilirubin remains stable for many years and a second phase of rapidly increasing values, the so called 'acceleration phase'. Repeated measurements of serum bilirubin >2.0mg/dl was a sign of late stage disease and preceded death within a few years.[97] A French study showed that persistent abnormal bilirubin levels were predictive for extensive fibrosis, with a positive predictive value of 90%.[83] In patients in whom serum bilirubin normalizes upon treatment with UDCA, transplant-free survival was found to be comparable with that in placebo-treated patients with initial normal serum bilirubin levels.[98] The same applied to survival of patients without normalization of bilirubin and placebo-using patients with abnormal bilirubin values at baseline. In other words, serum bilirubin values retain prognostic utility irrespective of treatment, underlining the utility of serum bilirubin as a useful surrogate endpoint of outcome.

Albumin is regarded as another important and powerful biochemical predictor of liver decompensation. Both, low serum albumin and high bilirubin values were shown to be independent predictors of the development of cirrhosis[83] and mortality.[70]

Angulo and colleagues were the first to report on the prognostic impact of changes in alkaline phosphatase values upon treatment with UDCA, showing that alkaline phosphatase values ≥2-fold the upper limit of normal (ULN) after 6 months of treatment predicted future treatment failure.[99] Several recent studies have also clearly demonstrated that quantitative decreases in bilirubin, albumin, alkaline phosphatase, aspartate aminotransferase (AST) and/or γ-GT levels after 6 months, 1 year or 2 years UDCA treatment, are predictive for improved transplant-free survival (**Table 2**). Responders according to these criteria were likely to have survival rates comparable with a general population. These biochemical response criteria are useful and now generally accepted tools for stratification purposes and for identifying patients in need of additional treatment.

Table 2. Biochemical response criteria for risk stratification in UDCA-treated primary biliary cirrhosis patients

Criteria[ref]	Definition of biochemical response	Evaluation time point	N
Mayo, 1999[99]	ALP <2.0xULN	6 months	180
Barcelona, 2006[38]	>40% decrease of ALP or normalization	1 year	192
Paris-1, 2008[39]	ALP <3.0xULN, AST <2.0xULN and bilirubin ≤1mg/dL	1 year	292
Rotterdam, 2009[40]	Normalization of abnormal bilirubin and/or albumin	1 year	375
Toronto, 2010[41, 100]	ALP ≤1.67xULN	2 years	69
Paris-2[#], 2011[42]	ALP ≤1.5xULN, AST ≤1.5xULN and bilirubin ≤1mg/dL	1 year	165
Ehim[^], 2011[101, 102]	≥70% decrease of γ-GT	6 months	138
Momah/Lindor, 2011[103]	ALP ≤1.67xULN and bilirubin ≤1mg/dL	1 year	73
Trivedi, 2014[104]	Paris-I, Paris-II, Barcelona or Toronto criteria and APRI ≤0.54	1 year	386

Abbreviations: ALP, alkaline phosphatase; AST, aspartate transaminase; APRI, AST to platelet ratio index
[#]only early PBC patients, [^]Japanese population

Presence of symptoms at time of diagnosis

Risk stratification according to the presence of symptoms at time of diagnosis has been the subject of many studies over the past decades.[84, 105-110] Of note, most studies did not use validated symptom assessment measures, which is essential for assessing the impact of subjective parameters, such as fatigue or pruritus. Therefore interpretation of such studies may be difficult. Most studies have reported that asymptomatic patients have earlier histologic stage of disease compared with symptomatic patients, in addition to more favourable liver enzyme profiles and lower bilirubin and higher albumin levels.[111] Several studies showed that a substantial proportion of asymptomatic patients will develop symptoms over time.[87, 106, 109, 111, 112] The vast majority (95%) of asymptomatic patients followed for up to 20 years will become symptomatic.[28] Once symptoms appear, survival of initially asymptomatic patients is comparable with survival of patients who initially presented with symptoms.[108, 111] Therefore asymptomatic PBC patients rather appear to represent an earlier stage of the disease than a separate clinical entity.

Gender and age at time of diagnosis

Data on the prognostic significance of factors such as gender or age are scare. The low prevalence of male or young patients with PBC (e.g. <35 years) has limited the possibility of studying these factors. In a large population-based study from Canada a higher risk of worse outcome was reported for males with PBC compared with females.[75] However, importantly is has been observed by several studies that male patients appear to present in a later stage of disease and this might be an important prognostic confounder explaining differences in prognosis between males and females. A recent landmark study from the UK PBC consortium clearly showed the impact of important disease subgroups in a study cohort

including 2353 PBC patients.[113] Importantly, male patients were less likely to respond to UDCA treatment and were at higher risk of worse outcome. Another important finding was an inverse relationship between age and likelihood to respond to UDCA. Thus, gender and age appear to be important in predicting prognosis in PBC.

PREDICTION OF OUTCOMES

Liver transplant-free survival

The ability to reliably predict outcome in patients with PBC is critically important in clinical management and an essential requirement for patient counselling and timing of diagnostic procedures and therapeutic interventions. Mathematical prediction models, either time-fixed or time-dependent, have been developed to predict the probability of survival using biochemical, clinical and/or histological features. Serum bilirubin and age are the main components of almost all proposed models.[79, 84, 114-117]

In the early eighties Roll et al. showed that age at time of diagnosis, presence of hepatomegaly and increased serum bilirubin were all independently associated with survival.[84] Notably, portal fibrosis was an independent predictor of prolonged survival in this study. Other studies identified (log)bilirubin,[107, 117, 118] variceal bleeding[107] albumin, age and ascites[118] as independent predictors of outcome. In a later study Bonsel et al. constructed a prognostic model incorporating nine variables: log(bilirubin), age, albumin, HBsAg, neurological complications, varices, ascites, clinical icterus and Quick-time prolongation.[115]

Two well defined and cross-validated models are the European Model and the Mayo risk score. The European model was published in 1985 by Christensen et al. based on data from 248 patients, originally included in an azathioprine placebo-controlled trial.[79] This time-fixed model included age at time of diagnosis, bilirubin, albumin, cirrhosis, central cholestasis and usage of azathioprine at baseline. In 1993 this group published two time-dependent models; one included only clinical and biochemical variables (bilirubin, ascites, albumin, age and gastrointestinal bleeding) and one extended version included additionally IgM and two histological variables (central cholestasis and cirrhosis). On the other hand, the Mayo risk score is the most frequently used model in PBC to predict short-term survival probability. This model was published in 1989 and cross-validated in independent cohorts.[114, 119] The following clinical and biochemical variables were included: age, serum bilirubin, serum albumin, prothrombin time (PT) and severity of edema. A great advantage of this model was that liver histology was not required to calculate the risk score. The original model was based on baseline characteristics and less useful to predict survival over time. An adapted Mayo model was proposed in 1994 using the same variables (INR instead of PT) to predict short-term (<2 years) survival or time to transplantation at any time point during follow-up.[120]

Data on the predictive value of the Mayo score after the introduction of UDCA treatment is conflicting. Kilmurry et al. showed that in a group of 222 patients originally included in an UDCA trial, the Mayo model remained a useful tool for prediction of survival when calculations are repeated after 6 months treatment.[121] Later studies suggested that the Mayo score overestimated the risk of death when applied before the start of treatment.[70, 72, 122] In a general sense the Mayo Risk Score is a useful tool to stratify patients for survival and possibly for clinical trials. A simplified model of the Mayo model was proposed by Kim et al.,[123] and web based applications are available for the Mayo model, which facilitate its usage in clinical practice.

In addition, more general prediction liver scores are used in PBC, such as the Model of End-Stage Liver Disease (MELD) score and the Child-Turcotte-Pugh-score.[124, 125] The MELD score is based on serum bilirubin, serum creatinine and INR. This score was originally proposed as a prognostic marker for the outcome after placement of a transjugular intrahepatic portosystemic shunt (TIPSS),[126] and currently used for liver organ allocation. We believe that the MELD score does not perform well in PBC and may result in excessive waiting time.

Portal hypertension and esophageal varices

Esophageal varices may develop in the cirrhotic and pre-cirrhotic stages of PBC.[68, 69] Survival of PBC patients who develop esophageal varices has been reported to be poor.[127, 128] Patanwala et al. reported a 5-year survival rate of 63% and 91% for patients with and without esophageal varices, respectively. The poor prognosis associated with esophageal varices may partly reflect the advanced stage of the disease in the majority of cases who develop varices, but may also be related to mortality associated with variceal bleeding. Therefore tools for timely diagnosis of varices and institution of prophylactic treatment are of obvious clinical importance.

A Mayo risk score ≥4.0 was seen in 93% of patients who developed esophageal varices,[99] while another study identified a Mayo risk score ≥4.5 together with a platelet count of <140.000/mm as independent risk factors for development of esophageal varices.[129] The current AASLD guideline on PBC recommends surveillance for esophageal varices of patients with a platelet count of <140.000/mm^3 or Mayo risk score >4.1.[33]

Recently the Newcastle Varices in PBC Score was proposed to predict esophageal varices,[128] based on a retrospective study including 330 PBC patients. This score was validated externally in two independent cohorts. Low albumin, low platelet count, abnormal alkaline phosphatase values and splenomegaly were independent predictors of varices development. An adapted score was proposed excluding splenomegaly to improve the usability in clinical practice and an online calculator is available (http://www.uk-pbc.com/resources/uk-pbc-varice-prediction-tool.html).

Prediction of hepatocellular carcinoma (HCC)

Primary liver cancer is a top five cause of cancer in men versus seventh in women and one of the most common causes of cancer death worldwide. HCC is most common form of liver cancer. HCC usually arises in patients with underlying chronic liver diseases, such as viral infections with chronic hepatitis B and C and alcohol hepatitis.[130] A recent systematic review and meta-analysis demonstrated a pooled relative risk of the development of HCC of 18.80 (95% CI, 10-81-26.79) for PBC patients compared with a general population, making HCC the most prevalent cancer in PBC.[131] The outcome of patients with HCC is poor.

HCC is less frequently seen in patients who initially present with early stage disease.[132, 133] Jones et al. followed-up 667 patients with early (stage I or II) and late (stage III or IV) stage disease, and both groups over the same period of time. All 16 HCC cases in this study were found in patients with advanced disease (stage III or IV) and not in patients with early disease (stage I or II).[134] A similar finding was reported by Floreani et al.[135] Additional Greek and Dutch studies clearly showed that despite the differences in disease stages at baseline, all HCC cases had advanced disease at time of HCC diagnosis.[40, 136] However, a study from Japan of 178 HCC cases, described HCC cases among all four histological stages,[137] especially in males. Histological stage at time of PBC diagnosis was independently associated with development of HCC for females, but not for males. These findings suggest that once cirrhosis occurs, risk of HCC development increases for females, but males may be at risk at any histological stage of disease. The Japanese study also showed a 10-year incidence of HCC for males versus females of 6.5% versus 2.0% ($P < 0.0001$). Several other studies also have demonstrated that in general males are more likely to develop HCC than females.[132-134] Estrogens are considered as having possibly protective effect on HCC development.

Male gender and advanced disease are the most frequently reported risk factors for HCC in PBC (**Table 3**). Suzuki et al. proposed a highly accurate prediction model (area under the curve of 0.95) to predict development of HCC. Patients with older age, male sex, history of blood transfusion and any signs of portal hypertension or cirrhosis were more likely to develop HCC.[133] These intriguing results await confirmation by other studies. Recently, absence of biochemical response in UDCA-treated PBC patients was proposed as another important risk factor for HCC. [138]

Surveillance strategies resulting in early diagnosis of HCC may improve outcome. Clearly, routine screening of all PBC patients on a regular basis is not practical. The current AASLD PBC guideline suggests that surveillance of HCC in PBC should be performed in cirrhotic patients and older men.[33] Possibly, the recently reported overwhelming prognostic importance of biochemical response to UDCA may prompt future modifications of present guidelines.

Table 3. Studies describing risk factors for the development of hepatocellular carcinoma in primary biliary cirrhosis

Group[ref], year	Study period	Type of study	N	HCC	incidence	Risk factors
Jones,[134] 1997	1975-1995	Population-based follow-up cohort study	667	16	2.4%	Male sex, presence of cirrhosis
Floreani,[135] 1999	1973-1996	Single-center follow-up cohort study	175	4	2.3%	History of cigarette smoking and HCV-RNA positivity
Caballería,[139] 2001	1977-1996	Single-center follow-up cohort study	140	5	3.6%	Advanced disease
Shibuya,[132] 2002	1980-1998	Multi-center prospective follow-up cohort	396	14	3.5%	Age at time of diagnosis, male sex, history of blood transfusion
Suzuki,[133] 2007	1976-2002	Case-control study	NA	17	-	Older age, male sex, blood transfusion, signs of portal hypertension or presence of cirrhosis
Silveira,[140] 2008	1976-2007	Single-center	NA	36	-	Age >70 years, blood transfusion, male gender, portal hypertension
Deutsch,[136] 2008	1987-2005	Single-center prospective cohort study	212	8	3.8%	Presence of cirrhosis and age
Cavazza,[141] 2009	-	Two-center follow-up cohort study	716	24	3.4%	Advanced histological stage
Kuiper,[138] 2010	1990-2007	Multi-center prospective cohort study	375	9	2.4%	Biochemical non-response to UDCA
Hosonuma, 2013			179	13	7.3%	Low levels of albumin
Harada,[137] 2013	1980-2009	National Survey of PBC patients	2946	71	2.4%	Advanced histological stage in females no independent risk factors in males
Harada,[137] 2013	2011	National Survey of PBC patients with HCC	NA	178	-	Advanced histological stage in females no independent risk factors in males
Tomiyama, 2013		Multicenter	210	11	5.2%	Advanced histological stage

OUTLINE OF THE THESIS

This thesis focuses on risk stratification in PBC. Studies included in this thesis address surrogate endpoints, biochemical response to UDCA treatment, and risk factors for mortality and liver cancer.

Chapter 1 provides an introduction to the epidemiology, pathogenesis, clinical presentation, diagnosis, treatment, prognosis and the prediction of outcomes in PBC.

Chapters 2.1 and 2.2 focus on surrogate endpoints in PBC. PBC is a rare and chronic liver disease, that slowly progresses to cirrhosis if untreated. UDCA, the only available medical treatment for PBC, has markedly improved the expected transplant-free survival probability, however, a substantial number of patients have an insufficient response to UDCA and these patients have an unmet medical need for new therapies. Presently, new therapies are under evaluation, however, it is debated what study endpoints should be used. Early clinical trials of UDCA evaluated clinical endpoints such as liver transplantation and death, but most cases of PBC are now diagnosed at an earlier stage of disease and UDCA therapy is initiated shortly after diagnosis, further affecting the ability to assess the clinical benefit of new therapies in a timely and realistic manner. Thus, surrogate endpoints of clinical outcomes are urgently needed to evaluate treatments effects. In **chapter 2.1 and 2.2** we assess the utility of the non-invasive biochemical variables serum alkaline phosphatase and bilirubin as robust surrogate endpoints of liver transplant-free survival. Their association, alone and in combination, with liver transplant-free survival is tested in different settings.

Chapters 3 and 4 deal with the significance of therapeutic response to UDCA and methods to quantify this response. Reliable identification of patients with an inadequate response to UDCA is of obvious key importance in clinical management, in particular for selecting those individuals who could benefit from additional, second-line medical therapies, but equally of those in whom UDCA mono-therapy can safely be continued. Over the past years several stratification tools using biochemical variables have been identified to identify high- and low-risk patients early in the treatment course. However, it is unclear how this concept of biochemical response to UDCA is used to guide further decision-making. **Chapter 3** contains a Dutch nationwide study exploring the impact of the concept of biochemical treatment response to UDCA in daily practice. Current biochemical response criteria have limitations to accurately identify PBC patients with an insufficient response to UDCA. In **chapter 4** a new stratification tool is presented based on non-invasive biochemical and clinical variables for early identification of patients with an insufficient treatment response to UDCA.

HCC is an infrequent, but critical event in PBC. Given its relative rarity, robust risk assessment has remained a challenge and current guidelines are mainly based on relatively small, single-centre studies. **Chapter 5** shows the results of a multicentre international cohort study assessing incidence and risk factors for HCC in PBC.

PBC predominantly affects middle-aged females. Considering its low prevalence, limited data is available on specific subgroups, such as male patients and young patients. Therefore, the aim of **chapter 6** is to identify sex and age differences in biochemical response and transplant-free survival among PBC patients.

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CHAPTER 2.1

Levels of alkaline phosphatase and bilirubin are surrogate endpoints of outcomes of patients with primary biliary cirrhosis: an international follow-up study

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Gastroenterology 2014; 147: 1338-1349.

ABSTRACT

Background & Aims: Non-invasive surrogate endpoints of long-term outcomes of patients with primary biliary cirrhosis (PBC) are needed to monitor disease progression and evaluate potential treatments. We performed a meta-analysis of individual patient data from cohort studies to evaluate whether patients' levels of alkaline phosphatase and bilirubin correlate with their outcomes and can be used as surrogate endpoints.

Methods: We performed a meta-analysis of data from 4845 patients included in 15 North American and European long-term follow-up cohort studies. Levels of alkaline phosphatase and bilirubin were analyzed in different settings and subpopulations at different time points relative to the clinical endpoint (liver transplantation or death).

Results: Of the 4845 patients, 1118 reached a clinical endpoint. The median follow-up period was 7.3 years; 77% survived for 10 years after study enrollment. Levels of alkaline phosphatase and bilirubin measured at study enrollment (baseline) and each year for 5 years were strongly associated with clinical outcomes (lower levels were associated with longer transplant-free survival). At 1 year after study enrollment, levels of alkaline phosphatase that were 2.0 times the upper limit of normal (ULN) best predicted patient outcome (C statistic, 0.71) but not significantly better than other thresholds. Of patients with alkaline phosphatase levels ≤2.0 times the ULN, 84% survived for 10 years compared with 62% of those with levels >2.0 times the ULN ($p<.0001$). Absolute levels of alkaline phosphatase 1 year after study enrollment predicted patient outcomes better than percentage change in level. One year after study enrollment, a bilirubin level 1.0 times the ULN best predicted patient transplant-free survival (C statistic, 0.79). Of patients with bilirubin levels ≤1.0 times the ULN, 86% survived for 10 years after study enrollment compared with 41% of those with levels >1.0 times the ULN ($p<.0001$). Combining levels of alkaline phosphatase and bilirubin increased the ability to predict patient survival times. We confirmed the predictive value of alkaline phosphatase and bilirubin levels in multiple subgroups, such as patients who had not received treatment with ursodeoxycholic acid, and at different time points after study enrollment.

Conclusions: Levels of alkaline phosphatase and bilirubin can predict outcomes (liver transplantation or death) of patients with PBC and might be used as surrogate endpoints in therapy trials.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a rare, chronic, and slowly progressive autoimmune hepatobiliary disease. PBC typically progresses to cirrhosis, which may lead to complications from liver failure and premature death.[1] Presently, most patients with PBC are treated with ursodeoxycholic acid (UDCA), the only approved therapy for PBC, which is in keeping with treatment guidelines.[2, 3] Although UDCA therapy has a marked impact on clinical outcomes in patients with PBC, up to 40% of patients have an insufficient response to this treatment and accordingly have a significantly increased risk of developing an adverse outcome, such as liver transplantation or death.[4-8] Therefore, there is a pressing unmet medical need for better therapies for this serious disease.

A major challenge for patients, health care providers, and drug developers is the slowly progressive nature of PBC, which effectively precludes the evaluation of classic clinical outcomes such as transplant-free survival. The low prevalence of PBC also represents a significant barrier to conducting large controlled clinical outcome trials in patients with this disease. Clinical endpoints such as liver transplantation and death were evaluated in an early primary interventional trial of UDCA in patients with PBC,[9] but most cases of PBC are now diagnosed at an earlier stage of disease and UDCA therapy is initiated shortly after diagnosis, further affecting the ability to assess the clinical benefit of new PBC therapies in a timely and realistic manner. Thus, the evaluation of scientifically valid surrogate parameters for clinical outcomes is inevitable at least at some stage in the development pathway. Further evaluation of possible surrogates for clinical benefit are needed, particularly with a focus on using large data sets that are representative of the spectrum of disease globally and sufficiently powered through size, duration of follow-up, and numbers of clinical events to refine the scientific validity of specific biochemical surrogates.

Serum bilirubin is well established as an independent predictor of prognosis in PBC, regardless of treatment.[10-12] In addition, bilirubin has previously been shown to be predictive of clinical outcomes across other liver diseases and is incorporated in several commonly used prognostic scoring models, such as the Child–Turcotte–Pugh score,[13, 14] the Model of End-Stage Liver Disease (MELD),[15] and, specifically in PBC, the Mayo PBC score.[16] However, despite the proven prognostic value of bilirubin, only patients with relatively advanced disease are likely to show meaningful changes in bilirubin levels that are stratifying. A biochemical variable and potentially more broadly applicable surrogate endpoint is alkaline phosphatase, an isoenzyme involved in dephosphorylation.[17] An elevated level of alkaline phosphatase, a marker of cholestasis, is typically seen across the spectrum of PBC disease severity and is a key component of the diagnosis of PBC in both the American and European guidelines.[2, 3] The relationship between alkaline phosphatase levels and the risk of adverse outcomes in PBC has been extensively documented in several relatively small studies,[4, 5,

[7, 8, 18, 19] but no systematic effort has been reported to date using a pooled meta-analysis approach to validate a biochemical surrogate for use in clinical studies of PBC.

We sought to investigate how serum alkaline phosphatase and bilirubin levels individually and in combination, correlate with transplant-free survival to determine the prognostic significance of these biochemical variables and hence their utility as robust surrogate endpoints for therapeutic PBC trials. To do so, we assembled a large, international, observational PBC database, allowing for a robust individual patient–level meta-analysis, to ensure both a rigorous statistical assessment and widespread applicability.

METHODS

Study design and study population

This study was a meta-analysis performed by the Global PBC Study Group, an international and multicenter collaboration between 15 liver centers in 8 North American and European countries, which combined individual patient data from major long-term follow-up cohorts. Most individual databases contained prospectively collected follow-up data on patients starting UDCA therapy.

This study was conducted in accordance with the protocol and the principles of the Declaration of Helsinki. The protocol was reviewed and approved by the institutional research board of the corresponding center and at each participating center in accordance with local regulations.

Both UDCA-treated and non-treated patients with an established diagnosis of PBC in accordance with European and American guidelines were eligible for inclusion in this study.[2, 3] Patients were excluded from analysis if follow-up data were insufficient or unavailable, the start date of treatment or the exact date of major clinical events was unknown, or they had concomitant liver disease.

Data collection and quality assessment

Collected clinical and laboratory data included sex, age, diagnosis of PBC, liver histology, treatment (type of medication, dosage, and duration), duration and last date of follow-up, baseline antimitochondrial antibody status, baseline and yearly laboratory levels (serum alkaline phosphatase, total bilirubin, albumin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, and platelets), and outcomes (death and cause of death, liver transplantation, hepatocellular carcinoma, ascites, and variceal bleeding).

Liver histology performed within 1 year of study entry or documented cirrhosis before study entry was classified as a baseline biopsy. Histological data was assessed for severity according to Ludwig[20] and Scheuer's[21] classification. Disease stage was classified

histologically as early (stage I and II) or late (stage III or IV) and biochemically using serum albumin and bilirubin levels. According to this biochemical classification, early stage was defined by normal bilirubin and albumin levels, moderately advanced disease was defined by an abnormal bilirubin or albumin level, and advanced disease was defined by abnormal bilirubin and albumin levels.[22]

Completeness, plausibility, and validity of the data were carefully verified. Extensive efforts, including site visits with review of medical charts, were undertaken to retrieve missing data. Data of the original cohorts were collected through the end of December 2012.

Statistical analysis

Study entry was defined as the start date of UDCA therapy or the date of the first center visit for patients not treated with UDCA. The primary endpoint was defined as a composite of either liver transplantation or death. Patients without documented events during follow-up were censored at their last follow-up visit.

To study the association between the absolute alkaline phosphatase and bilirubin levels, the hazard ratios (HRs) of liver transplantation or death were estimated by applying a cubic spline function of alkaline phosphatase and bilirubin at baseline and yearly up to 5 years of follow-up.

To find an optimal threshold for each variable, alkaline phosphatase and bilirubin levels at 1 year of follow-up were categorized according to multiple thresholds ranging from 1.0 to 3.0 times the upper limit of normal (ULN) in steps of 0.1 (including 1.67 times the ULN[7] for alkaline phosphatase levels).

The C statistic was calculated for each of these thresholds to evaluate their ability to predict liver transplant-free survival. Accompanying HRs were calculated for each threshold by using the Cox proportional hazard regression model. The log-likelihood test was used to assess significance. Transplant-free survival was assessed for the peak thresholds of alkaline phosphatase and bilirubin levels and for a combination of both by Kaplan–Meier estimates. Log-rank test was used for comparisons between groups.

In addition to the predictive ability of absolute levels of alkaline phosphatase, the percentage change in alkaline phosphatase levels[4] from baseline to 1-year follow-up was evaluated using the same approach.

All analyses were stratified by center to account for possible heterogeneity across center populations. The effects of alkaline phosphatase and bilirubin were adjusted for year of diagnosis, age at study entry, UDCA therapy, and sex.

To investigate if alkaline phosphatase and bilirubin levels are meaningful surrogate endpoints, the association with the clinical endpoint must hold true independent of time and specific patient subgroups. Therefore, the survival analyses were repeated at different

time points and for multiple subgroups of patients. The time points analyzed were baseline and yearly up to 5 years of follow-up. Given the nature of this study, alkaline phosphatase or bilirubin levels were not always available for every patient at these time points. Accordingly, we aimed for the optimal use of the available data by assessing the association with hard clinical endpoints at baseline and several intervals thereafter up to 5 years. Subgroups were defined by treatment (UDCA-treated and non-treated patients), baseline alkaline phosphatase levels (>2.0 times the ULN and >4.0 times the ULN), baseline bilirubin levels (>1 times the ULN and >3 times the ULN), PBC disease state based on both histology and biochemistry, age at time of diagnosis (younger than 45 years and 45 years or older),[23] sex, and date of diagnosis (before 1990, 1990–1999, and 2000–2009).

Normally distributed data are presented as mean ± standard deviation (SD) and skewed distributed data as median and interquartile range. All analyses were 2 sided. $p<.05$ was considered statistically significant. Statistical analyses were performed with IBM SPSS Statistics 22.0 (SPSS Inc, Chicago, IL) and SAS 9.3 (SAS institute, Cary, NC).

RESULTS

Baseline data

Data were obtained from 6191 patients with PBC, of whom 4845 met the inclusion criteria (**Figure 1**). A total of 65,642 patient visits and a mean of 11 visits per patient were reported across the entire cohort, with a median of 132 elapsing days between visits. Cohort characteristics per center are summarized in **Table 1**. The year in which PBC was diagnosed ranged from 1959 to 2012. The diagnosis was established after 1990 for 79% of patients, and the median year of diagnosis was 1998 (interquartile range, 1991–2004). The median follow-up period was 7.3 years (interquartile range, 3.6–11.5 years) for the cohort, ranging from 6 months to 34 years.

Table 1. Center specific characteristics of the study population

Center		Year of diagnosis		Follow-up (in years)		UDCA		Alkaline phosphatase at entry	Bilirubin at entry
Country (city)	N	median	Time frame	Median (IQR)	Range	N	%	Median (IQR)	Median (IQR)
USA, (*Rochester*)	857	2002	1970-2012	4.7 (2.3-9.0)	0.5-18.1	590	69%	1.63 (1.07-2.59)	0.80 (0.50-1.40)
The Netherlands, *(Nationwide cohort*)	838	1999	1961-2012	8.9 (4.8-14.2)	0.5-24.2	838	100%	2.10 (1.39-3.63)	0.61 (0.44-0.90)
Canada, (*Toronto*)	628	1999	1974-2010	7.3 (4.0-11.4)	0.5-34.3	529	84%	2.50 (1.66-4.60)	0.55 (0.40-0.82)
Italy, (*Padua*)	544	1989	1959-2005	7.1 (3.6-12.0)	0.5-25.8	386	71%	2.56 (1.50-4.29)	0.80 (0.54-1.38)
UK, (*Birmingham*)	363	2003	1972-2011	6.0 (3.3-9.4)	0.6-16.7	285	79%	1.91 (1.16-3.20)	0.52 (0.38-1.10)
French, (*Paris*)	348	1988	1974-2001	5.9 (2.1-8.9)	0.5-22.5	348	100%	3.00 (1.90-5.30)	0.67 (0.43-1.17)
USA, (*Dallas*)	326	1993	1977-2011	8.8 (6.9-11.6)	0.8-23.7	326	100%	2.67 (1.54-3.86)	0.46 (0.31-0.67)
Italy, (*Milan, 2 centers*)	289	1999	1972-2012	7.2 (3.4-13.3)	0.5-23.8	289	96%	1.74 (1.05-3.26)	0.67(0.48-1.00)
Spain, (*Barcelona*)	273	1995	1971-2005	12.1 (7.7-16.3)	0.6-23.8	266	97%	1.89 (1.24-3.32)	0.67 (0.50-1.00)
Belgium, (*Leuven*)	150	2000	1974-2011	6.8 (3.4-12.8)	0.6-28.8	136	91%	2.75 (1.66-4.58)	0.72 (0.47-1.18)
UK, (*London*)	138	1994	1972-2007	8.5 (5.1-12.1)	0.5-22.5	56	41%	1.83 (1.14-3.09)	0.53 (0.41-0.71)
Canada, (*Edmonton*)	57	2003	1989-2008	5.9 (4.0-8.3)	0.7-18.4	53	93%	3.13 (2.10-5.57)	0.82 (0.57-1.24)
USA, (*Seattle*)	34	2008	1995-2012	6.0 (3.3-9.4)	0.5-16.7	30	88%	1.69 (1.11-2.68)	0.42 (0.33-0.50)
Total	4845	1998	1959-2012	7.3 (3.6-11.5)	0.5-34.3	4119	85%	2.10 (1.31-3.72)	0.67 (0.45-1.06)

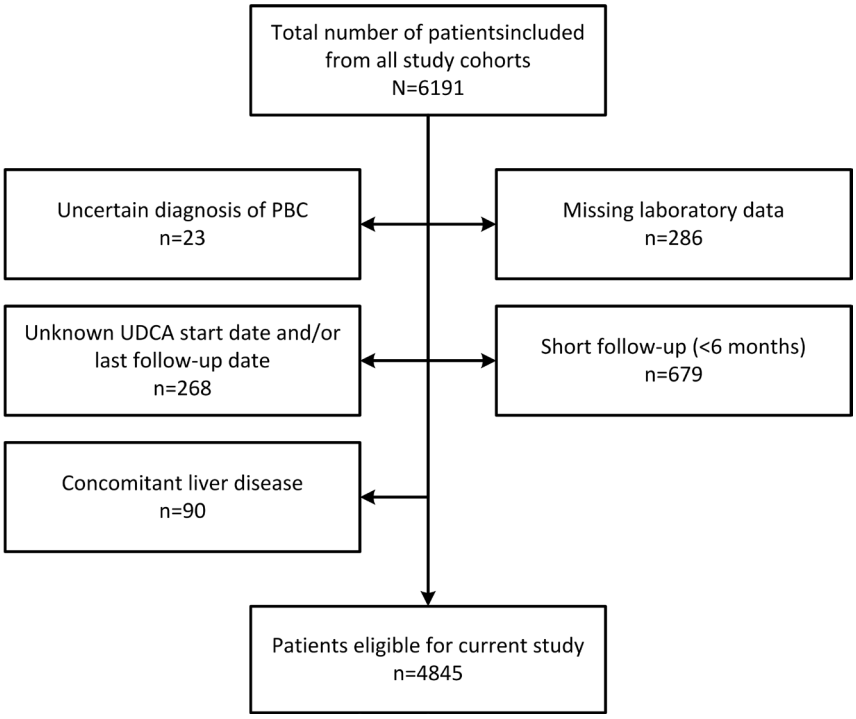

Figure 1. Flow chart
Flow diagram of patients included in this study.

Clinical and biochemical patient characteristics are shown in **Table 2**. Overall, the demographics were consistent with previous reports of PBC disease epidemiology. Most patients (4119 [85%]) were treated with UDCA at a median dosage of 12.3 $mg*kg^{-1}*day^{-1}$ (interquartile range 9.4–14.6 $mg*kg^{-1}*day^{-1}$). Histological stage of disease was available for 76% of patients who had undergone a liver biopsy; most had a diagnosis of early disease (stage I or II).

During follow-up, 1118 patients reached a clinical endpoint; 389 underwent liver transplantation and 729 died; 358 (49%) died of liver-related causes, 245 patients (34%) died of other causes, and the cause of death was unknown for 126 patients (17%). In the total cohort, 5-year transplant-free survival was 88%, 10-year survival was 77%, and 15-year survival was 63%; in UDCA-treated patients, these findings were 90%, 78%, and 66%, respectively, and in non-treated patients 79%, 59%, and 32%, respectively, (treated vs non-treated, $p<.0001$).

The effects of factors adjusted for in further analyses are shown in **Table 3**.

Table 2. Baseline patient characteristics

	Total cohort (n = 4845)
Age at entry (years)[a]	54.5 (12.0)
Female, n (%)	4348 (90%)
AMA+, n (%)	4280 (88%)
Year of diagnosis[b]	1998 (1991-2004)
Year of diagnosis, time frame	1959-2012
Histological disease stage, n (%)[c]	
Stage I	1017 (27%)
Stage II	862 (23%)
Stage III	483 (13%)
Stage IV	454 (12%)
Not available	953 (25%)
Biochemical disease stage, n (%)[d]	
Early	2040 (42%)
Moderately advanced	730 (15%)
Advanced	259 (5%)
Not available	1816 (38%)
Baseline alkaline phosphatase levels, n (%)	
>2.0xULN	1931 (52%)
>4.0xULN	816 (22%)
Not available	1140 (24%)
UDCA treated patients, n (%)[e]	4119 (85%)
Laboratory data at entry[b]	
Serum bilirubin (xULN)	0.67 (0.45-1.06)
Not available	1118 (23%)
Serum alkaline phosphatase (xULN)	2.10 (1.31-3.72)
Not available	1140 (24%)

Abbreviations: AMA, antimitochondrial antibody; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.
[a]Data is expressed as mean and standard deviation
[b]Data is expressed as median and interquartile range
[c]Histological disease stage according to Ludwig and Scheuer's classification
[d]Biochemical disease stage according to Rotterdam criteria (using albumin and bilirubin)[22]
[e]640 subjects were non-treated and 86 subjects were without definitive information on UDCA use

Table 3. Univariable and multivariable analysis showing the effects of variables at baseline predictive for liver transplantation and death

	Univariable analysis		Multivariable analysis	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Year of diagnosis (year)	0.95 (0.94-0.95)	<0.001	0.95 (0.94-0.96)	<0.001
Age at study entry (year)	1.04 (1.03-1.04)	<0.001	1.03 (1.03-1.04)	<0.001
UDCA treatment	0.59 (0.50-0.71)	<0.001	0.61 (0.51-0.74)	<0.001
Male gender	1.52 (1.28-1.80)	<0.001	1.46 (1.22-1.75)	<0.001

The association between alkaline phosphatase and bilirubin levels and the risk of liver transplantation or death

A log-linear association was observed between alkaline phosphatase levels and the risk of liver transplantation and death after 1 year and up to 5 years of follow-up, whereby higher alkaline phosphatase levels were associated with reduced transplant-free survival. This association was also found for baseline alkaline phosphatase levels, thus irrespective of subsequent UDCA therapy (**Figure 2A** and **Figure 3A**). Abnormal bilirubin levels were even more strongly associated with poor clinical outcome at baseline and up to 5 years of follow-up (**Figure 2B** and **Figure 3B**).

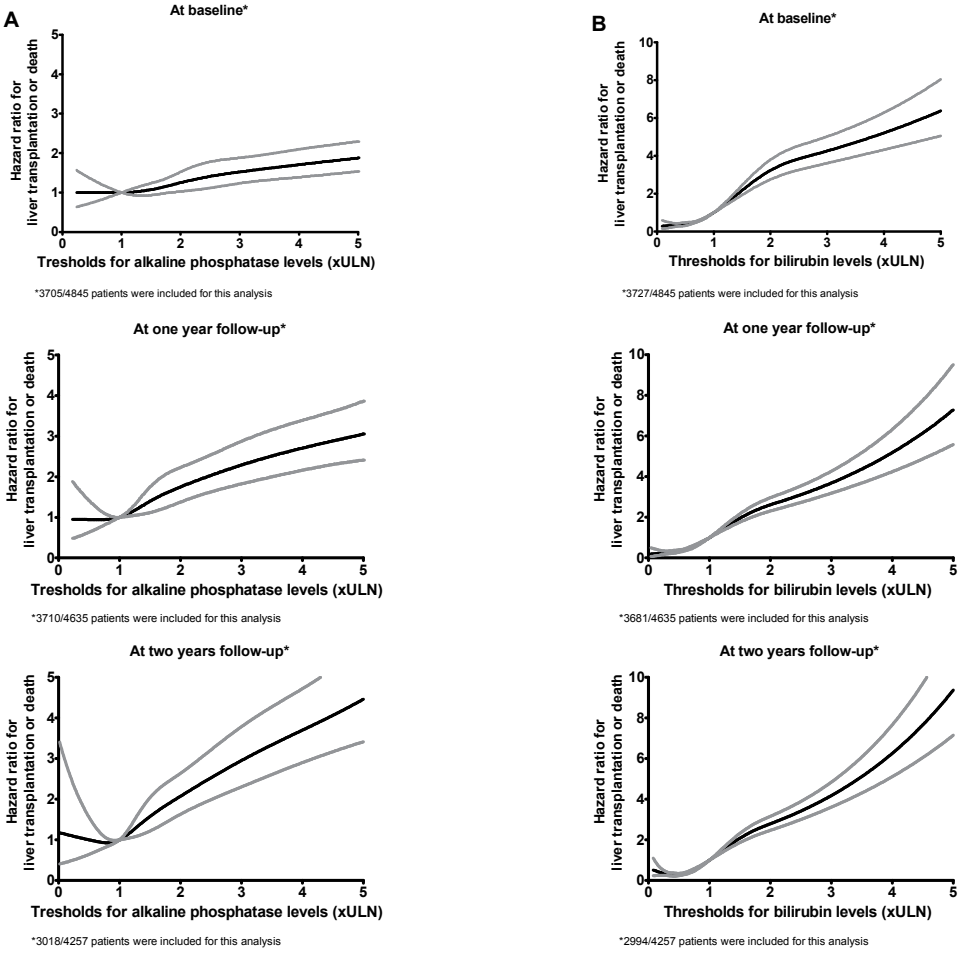

Figure 2. Hazard of liver transplantation or death for alkaline phosphatase and bilirubin levels
The hazard of liver transplantation or death for (A) alkaline phosphatase levels and (B) bilirubin levels at different time points estimated with cubic spline function.

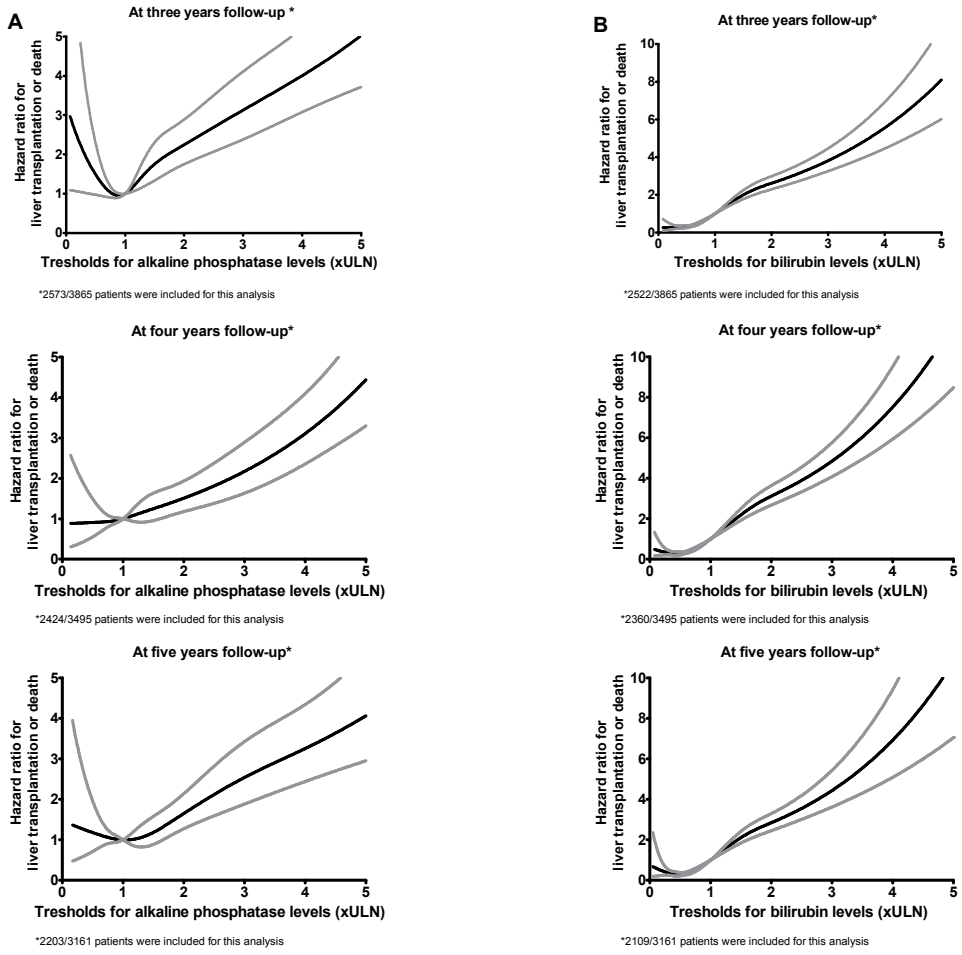

Figure 3. Hazard of liver transplantation or death for alkaline phosphatase and bilirubin levels
The hazard of liver transplantation or death for (A) alkaline phosphatase levels and (B) bilirubin levels at different time points estimated with cubic spline function.

Optimal threshold for alkaline phosphatase and bilirubin levels and the risk of liver transplantation and death

The study population was analyzed according to a multitude of thresholds for alkaline phosphatase levels at 1 year of follow-up. This analysis consistently showed that patients with alkaline phosphatase levels below any of these thresholds had significantly improved transplant-free survival compared with patients with alkaline phosphatase levels above the thresholds (**Table 4**).

Table 4. Performance of different alkaline phosphatase and bilirubin thresholds at one year follow-up for prediction liver transplantation or death

	Alkaline phosphatase (n=3710)				Bilirubin (n=3681)			
Thresholds	**C statistic (95% CI)**	**Hazard ratio (95% CI)**	**P-value**	**Number of patients ≤/> threshold**	**C statistic (95% C()**	**Hazard ratio (95% CI)**	**P-value**	**Number of patients ≤/> threshold**
1.0xULN	0.68 (0.66-0.70)	2.06 (1.69-2.52)	<.001	1071/2639	0.79 (0.77-0.80)	5.06 (4.34-5.89)	<.001	2941/740
1.1xULN	0.69 (0.67-0.71)	2.14 (1.79-2.57)	<.001	1306/2404	0.78 (0.77-0.80)	5.22 (4.48-6.08)	<.001	3019/662
1.2xULN	0.69 (0.67-0.71)	1.97 (1.66-2.33)	<.001	1515/2195	0.78 (0.76-0.80)	5.95 (5.09-6.94)	<.001	3108/573
1.3xULN	0.69 (0.67-0.71)	2.02 (1.72-2.37)	<.001	1727/1983	0.78 (0.76-0.80)	6.58 (5.61-7.72)	<.001	3172/509
1.4xULN	0.70 (0.68-0.71)	2.05 (1.75-2.39)	<.001	1900/1810	0.78 (0.76-0.80)	6.87 (5.84-8.09)	<.001	3219/462
1.5xULN	0.70 (0.68-0.72)	2.14 (1.84-2.50)	<.001	2030/1680	0.77 (0.76-0.79)	7.68 (6.47-9.12)	<.001	3271/410
1.6xULN	0.70 (0.69-0.72)	2.23 (1.92-2.60)	<.001	2158/1552	0.77 (0.75-0.79)	8.32 (6.99-9.91)	<.001	3297/384
1.67xULN	0.70 (0.69-0.72)	2.18 (1.88-2.53)	<.001	2231/1479				
1.7xULN	0.70 (0.69-0.72)	2.22 (1.91-2.57)	<.001	2274/1436	0.77 (0.75-0.79)	8.99 (7.53-10.74)	<.001	3323/358
1.8xULN	0.71 (0.69-0.73)	2.31 (1.99-2.68)	<.001	2393/1317	0.77 (0.75-0.78)	9.04 (7.53-10.84)	<.001	3346/335
1.9xULN	0.71 (0.69-0.73)	2.37 (2.04-2.75)	<.001	2466/1244	0.76 (0.75-0.78)	9.30 (7.73-11.20)	<.001	3368/313
2.0xULN	0.71 (0.69-0.73)	2.49 (2.14-2.89)	<.001	2571/1139	0.76 (0.74-0.78)	10.33 (8.50-12.54)	<.001	3404/277
2.1xULN	0.71 (0.69-0.72)	2.41 (2.07-2.80)	<.001	2648/1062	0.76 (0.74-0.78)	10.66 (8.76-12.97)	<.001	3417/264
2.2xULN	0.70 (0.69-0.72)	2.38 (2.05-2.77)	<.001	2714/996	0.75 (0.73-0.77)	10.31 (8.43-12.62)	<.001	3439/242
2.3xULN	0.70 (0.68-0.72)	2.37 (2.04-2.76)	<.001	2774/936	0.75 (0.73-0.77)	9.98 (8.13-12.24)	<.001	3449/232
2.4xULN	0.70 (0.68-0.72)	2.37 (2.04-2.77)	<.001	2831/879	0.74 (0.73-0.76)	10.43 (8.46-12.86)	<.001	3461/220
2.5xULN	0.70 (0.68-0.72)	2.31 (1.98-2.70)	<.001	2885/825	0.74 (0.72-0.76)	10.08 (8.14-12.50)	<.001	3473/208
2.6xULN	0.70 (0.68-0.72)	2.40 (2.05-2.81)	<.001	2934/776	0.73 (0.71-0.75)	9.81 (7.89-12.21)	<.001	3482/199
2.7xULN	0.69 (0.67-0.71)	2.38 (2.04-2.79)	<.001	2983/727	0.73 (0.71-0.75)	10.08 (8.07-12.59)	<.001	3487/194
2.8xULN	0.69 (0.67-0.71)	2.26 (1.92-2.65)	<.001	3036/674	0.73 (0.71-0.75)	9.80 (7.85-12.24)	<.001	3495/186
2.9xULN	0.69 (0.67-0.71)	2.32 (1.97-2.73)	<.001	3072/638	0.73 (0.71-0.75)	9.49 (7.57-11.91)	<.001	3507/174
3.0xULN	0.69 (0.67-0.71)	2.31 (1.96-2.73)	<.001	3104/606	0.72 (0.70-0.74)	9.10 (7.23-11.45)	<.001	3516/165

After 1 year of follow-up, while all thresholds were predictive of outcomes, a threshold of 2.0 times the ULN for alkaline phosphatase levels was found to have the highest predictive ability (C statistic, 0.71; 95% confidence interval [CI], 0.69–0.73). Notably, this threshold was not a significantly better predictor than the other thresholds, such as 1.5 times the ULN,[8] 1.67 times the ULN,[7, 19] or 3.0 times the ULN[5] (**Table 4** and **Figure 4**). Similarly, all assessed bilirubin thresholds were predictive of outcomes. For bilirubin, a threshold of 1.0 times the ULN had the highest predictive ability (C statistic, 0.79; 95% CI, 0.77–0.80) (**Table 4**).

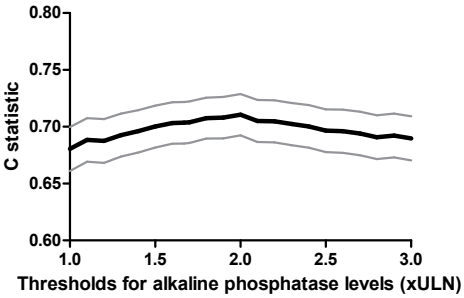

Figure 4. **Performance of alkaline phosphatase thresholds**
C statistic was performed for different thresholds for alkaline phosphatase levels at one year follow-up. The C statistic reflects the predictive accuracy of alkaline phosphatase thresholds to distinguish patients with a high risk of liver transplantation or death from patients with a low risk.

The 5-, 10-, and 15-year transplant-free survival rates for patients with alkaline phosphatase levels ≤2.0 times the ULN were 94%, 84%, and 73%, respectively; for patients with alkaline phosphatase levels >2.0 times the ULN, these rates were 81%, 62%, and 50%, respectively (p<.0001), as shown in **Figure 5A**. The accompanying 5-, 10-, and 15-year transplant-free survival rates for patients with normal bilirubin levels after 1 year of follow-up were 95%, 86%, and 74%, respectively; for patients with abnormal bilirubin levels these rates were 65%, 41%, and 30%, respectively (p<.0001) (**Figure 5B**).

The prognostic information provided by alkaline phosphatase levels remained important in addition to bilirubin levels; the risk of liver transplantation or death of patients with alkaline phosphatase levels >2.0 times the ULN was significantly higher in both those patients with normal (≤1 times the ULN) and abnormal bilirubin (>1 times the ULN) levels. The 5-, 10-, and 15-year transplant-free survival rates in the normal bilirubin group for patients with alkaline phosphatase levels ≤2.0 times the ULN were 97%, 89%, and 79%, respectively; for patients with alkaline phosphatase levels >2.0 times the ULN, these rates were 95%, 82%, and 68%, respectively (p<.0001). In the abnormal bilirubin group, these rates were 74%, 51%, and 39%, respectively, for patients with alkaline phosphatase levels ≤2.0 times the ULN and 63%, 34%, and 24%, respectively, for patients with alkaline phosphatase levels >2.0 times the ULN (p<.0001) (**Figure 5C**).

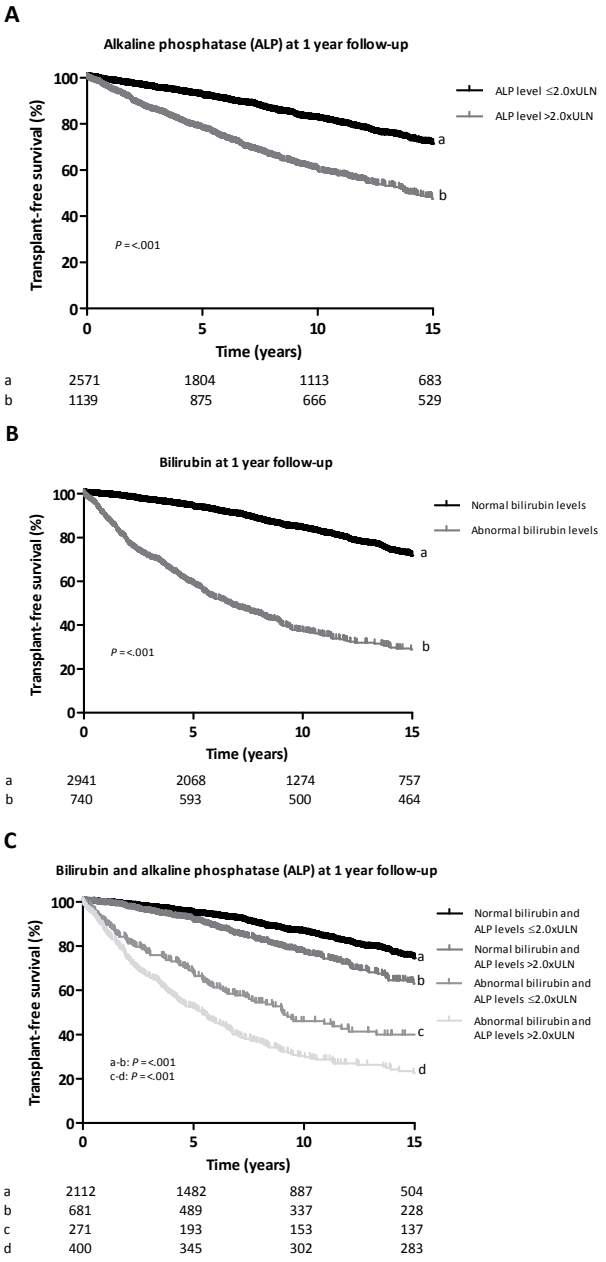

Figure 5. Liver transplantation-free survival

(A) Transplant-free survival of patients with alkaline phosphatase levels ≤2.0xULN versus >2.0xULN at one year follow-up. (B) Transplant-free survival of patients with bilirubin levels ≤1.0xULN versus >1.0xULN at one year follow-up. (C) Transplant-free survival of patients with alkaline phosphatase levels ≤2.0xULN versus >2.0xULN at one year follow-up within both, patients with bilirubin levels ≤1xULN and >1xULN.

An alkaline phosphatase threshold of 2.0 times the ULN was also predictive in addition to other bilirubin thresholds between 1.0 times and 3.0 times the ULN, but was not predictive in addition to bilirubin levels >3 times the ULN (HR, 0.71; 95% CI, 0.39–1.32; p=.29). Comparable results were found for other alkaline phosphatase thresholds (eg, 1.5 times the ULN and 1.67 times the ULN in combination with normal or abnormal bilirubin levels) (data not shown).

The predictive value of percentage changes in alkaline phosphatase levels at 1-year follow-up

A prior study showed that patients who achieved a normal alkaline phosphatase level or had a >40% decrease in alkaline phosphatase levels after UDCA therapy had a normal prognosis.[4] In line with this study, the percentage change in alkaline phosphatase levels from baseline to 1-year follow-up was predictive of outcome; the greater the percentage decrease in alkaline phosphatase levels, the better the transplant-free survival (HR per 10% change in alkaline phosphatase levels 0.98; 95% CI, 0.96–0.99; p<.01).

A >40% decrease in alkaline phosphatase levels was found to be significant in predicting outcome (**Table 5**). The predictive value of percentage decrease of alkaline phosphatase levels with UDCA therapy was independent of the baseline alkaline phosphatase levels in patients with a decrease between 0–40% and >40% compared with patients without any decrease (**Figure 6**).

However, the percentage decrease in alkaline phosphatase levels did not add prognostic information to absolute alkaline phosphatase levels after 1 year of follow-up (HR per 10% change in alkaline phosphatase levels 1.00; 95% CI, 0.99–1.02; p=.72), apart from very high alkaline phosphatase levels (>5.0 times the ULN) (HR per 10% change in alkaline phosphatase level, 0.86; 95% CI, 0.76–0.96; p<.005).

Table 5. Hazard ratio for predicting liver transplantation or death for percentage change of alkaline phosphatase levels from baseline to one year follow-up

Percentage reduction of alkaline phosphatase	Hazard Ratio (95% CI)	P-value
No reduction	1	
0-10%	0.88 (0.63-1.23)	.45
10-20%	0.85 (0.60-1.20)	.36
20-30%	0.67 (0.48-0.95)	.03
30-40%	0.84 (0.61-1.15)	.23
40-50%	0.70 (0.51-0.96)	.03
50-60%	0.59 (0.42-0.83)	.003
>60%	0.62 (0.44-0.86)	.005

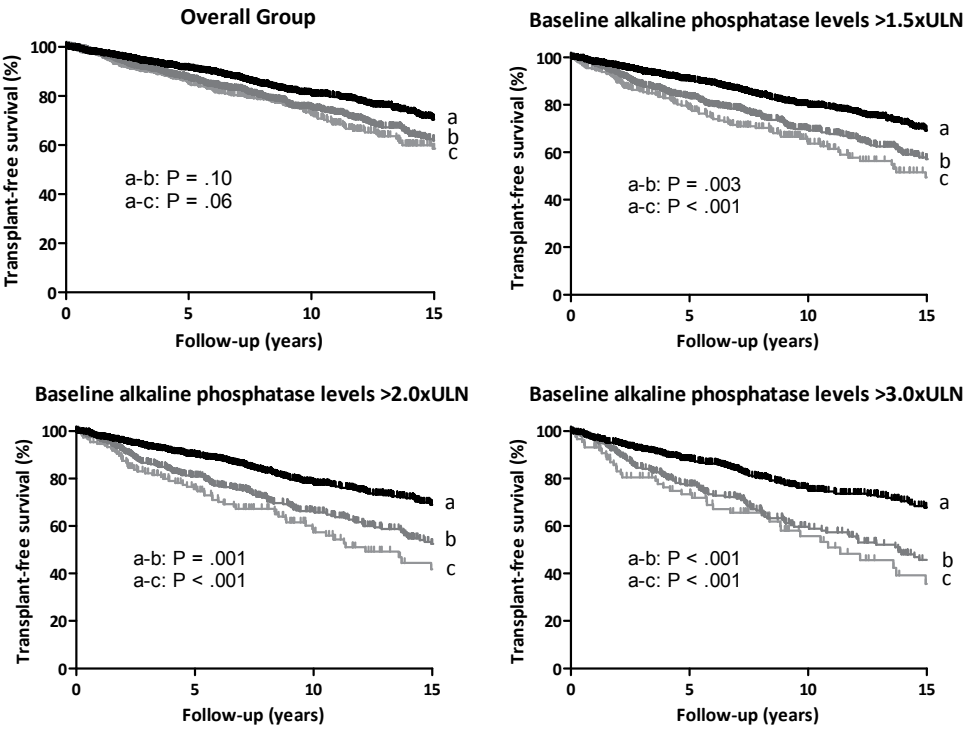

Figure 6. Liver transplant-free survival for percent decrease of alkaline phosphatase levels
Transplant-free survival for percent decrease of alkaline phosphatase levels at one year follow-up. (A) Transplant-free survival of patients with >40% decrease of alkaline phosphatase levels, (B) Transplant-free survival of patients with 0-40% decrease of alkaline phosphatase levels and (C) Transplant-free survival for patients with no decrease of alkaline phosphatase levels.

Predictive ability of alkaline phosphatase and bilirubin levels across subgroups

To assess if alkaline phosphatase can be used as a predictor independent of patient characteristics, the previously described analyses were repeated for a range of subgroups (**Figure 7A**). Of note, using an alkaline phosphatase threshold of 2.0 times the ULN after 1 year of follow-up was not only predictive for UDCA-treated patients but also for non-treated patients. Similar results were seen in patients with baseline alkaline phosphatase levels >2.0 times the ULN and >4.0 times the ULN, patients with histologically early and late disease, patients with biochemically early and moderately advanced disease, patients 45 years of age or younger at diagnosis and older than 45 years of age at diagnosis, male and female patients, and regardless of the year of diagnosis. Alkaline phosphatase levels were not predictive for patients with advanced biochemical disease (ie, patients with both abnormal bilirubin and albumin levels). A bilirubin threshold of 1.0 times the ULN after 1 year of follow-up was also predictive in several subsets of patients (**Figure 7B**).

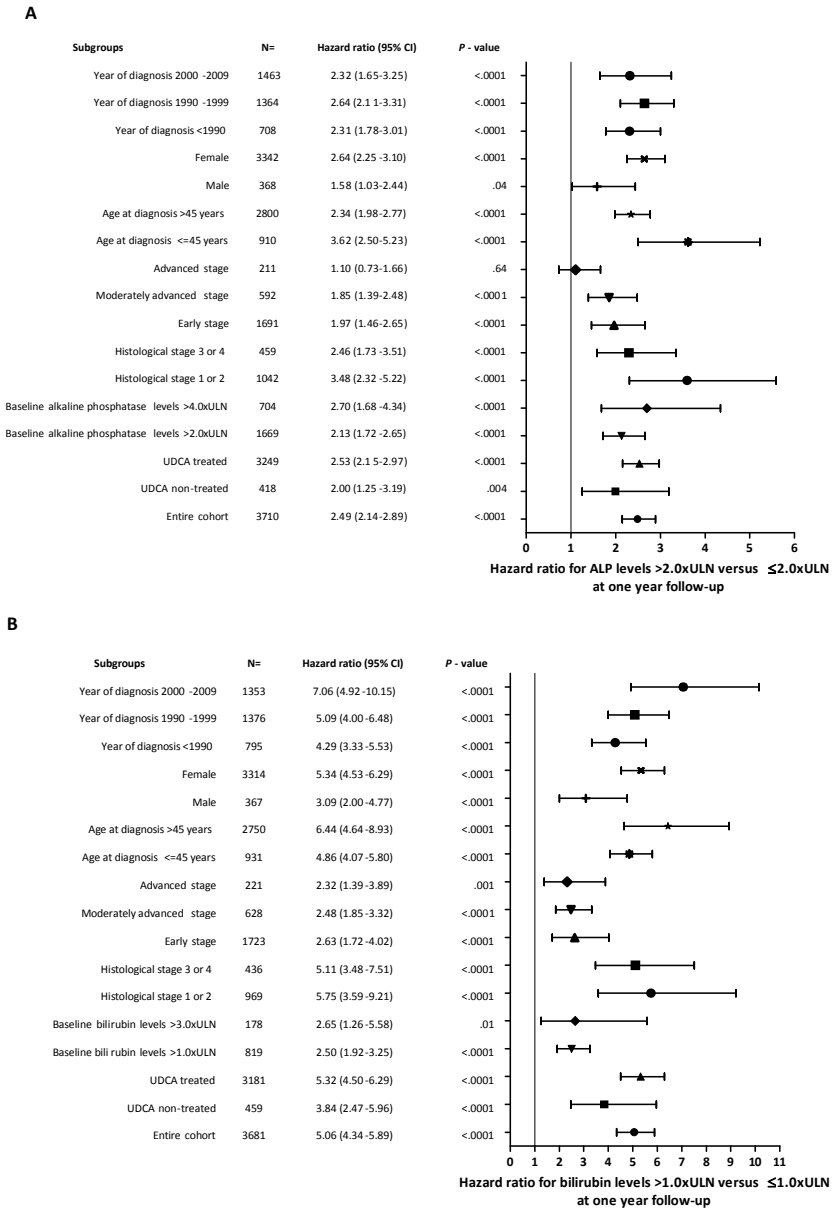

Figure 7. Subgroup analyses of alkaline phosphatase and bilirubin levels
Hazard ratio of liver transplantation or death for (A) alkaline phosphatase levels >2.0xULN versus ≤2.0xULN and (B) bilirubin levels >1.0xULN versus ≤1.0xULN at one year follow-up for different subgroups.

Comparable results were found for alkaline phosphatase and bilirubin at other time points among almost all subgroups (**Table 6**).

Table 6. Hazard ratio of liver transplantation or death for (A) alkaline phosphatase levels >2.0xULN versus ≤2.0xULN and (B) bilirubin levels >1.0xULN versus ≤1.0xULN at baseline and two years follow-up for different subgroups

	Alkaline phosphatase >2.0xULN versus ≤2.0xULN At baseline			Alkaline phosphatase >2.0xULN versus ≤2.0xULN At 2 years follow-up		
	n=	P-value	HR (95% CI)	n=	P-value	HR (95% CI)
Year of diagnosis 2000-2009	1479	<.0001	2.23 (1.65-3.02)	1170	<.0001	2.50 (1.61-3.88)
Year of diagnosis 1990-1999	1298	<.0001	2.05 (1.62-2.60)	1176	<.0001	2.41 (1.87-3.11)
Year of diagnosis <1990	754	.002	1.54 (1.17-2.04)	579	<.0001	2.41 (1.78-3.25)
Female	3320	<.0001	1.94 (1.65-2.29)	2717	<.0001	2.75 (2.27-3.32)
Male	385	.01	1.64 (1.11-2.42)	301	.64	1.13 (0.67-1.91)
Age at diagnosis >45 years	2847	<.0001	1.84 (1.56-2.17)	2264	<.0001	2.35 (1.93-2.87)
Age at diagnosis ≤45 years	858	<.0001	2.52 (1.65-3.84)	754	<.0001	3.11 (2.08-4.64)
Advanced stage	239	.44	1.17 (0.78-1.75)	152	.57	1.17 (0.68-2.01)
Moderately advanced stage	667	.79	1.04 (0.79-1.37)	453	.01	1.56 (1.11-2.19)
Early stage	1905	.002	1.52 (1.16-2.00)	1347	<.0001	2.02 (1.42-2.87)
Histological stage 3 or 4	449	.54	0.88 (0.59-1.32)	396	.0008	2.26 (1.40-3.64)
Histological stage 1 or 2	1013	<.0001	2.63 (1.73-3.99)	866	<.0001	4.15 (2.50-6.87)
Baseline alkaline phosphatase levels >4.0xULN				557	<.0001	2.87 (1.80-4.58)
Baseline alkaline phosphatase levels >2.0xULN				1342	<.0001	2.22 (1.74-2.83)
UDCA treated	3090	<.0001	2.01 (1.68-2.39)	2719	<.0001	2.68 (2.22-3.23)
UDCA non-treated	537	.003	1.68 (1.19-2.37)	265	.06	1.67 (0.97-2.86)
Entire cohort	3705	<.0001	1.87 (1.61-2.18)	3018	<.0001	2.49 (2.09-2.96)

Table 6 (continued). Hazard ratio of liver transplantation or death for (A) alkaline phosphatase levels >2.0xULN versus ≤2.0xULN and (B) bilirubin levels >1.0xULN versus ≤1.0xULN at baseline and two years follow-up for different subgroups

	Bilirubin >1.0xULN versus ≤1.0xULN At baseline			Bilirubin >1.0xULN versus ≤1.0xULN At 2 years follow-up		
	n=	P-value	HR (95% CI)	n=	P-value	HR (95% CI)
Year of diagnosis 2000-2009	1409	<.0001	4.73 (3.45-6.48)	1054	<.0001	6.55 (4.09-10.48)
Year of diagnosis 1990-1999	1312	<.0001	5.50 (4.36-6.93)	1194	<.0001	4.54 (3.46-5.96)
Year of diagnosis <1990	846	<.0001	4.00 (3.16-5.05)	665	<.0001	4.33 (3.22-5.82)
Female	3332	<.0001	4.94 (4.24-5.75)	2699	<.0001	4.92 (4.06-5.97)
Male	395	<.0001	3.50 (2.36-5.19)	295	<.0001	4.34 (2.55-7.38)
Age at diagnosis >45 years	901	<.0001	4.27 (3.64-5.01)	2225	<.0001	4.23 (3.44-5.21)
Age at diagnosis <≤45 years	2826	<.0001	7.25 (5.12-10.26)	769	<.0001	8.62 (5.79-12.83)
Advanced stage				161	.01	2.33 (1.20-4.51)
Moderately advanced stage				477	<.0001	2.49 (1.74-3.57)
Early stage				1379	<.0001	3.70 (2.42-5.67)
Histological stage 3 or 4	429	<.0001	3.80 (2.54-5.70)	370	<.0001	4.20 (2.73-6.48)
Histological stage 1 or 2	946	<.0001	8.98 (5.63-14.32)	814	<.0001	5.19 (3.08-8.74)
Baseline bilirubin levels >3.0xULN				116	.35	1.51 (0.64-3.53)
Baseline bilirubin levels >1.0xULN				630	<.0001	2.09 (1.54-2.84)
UDCA treated	3069	<.0001	5.28 (4.50-6.20)	2662	<.0001	5.05 (4.17-6.13)
UDCA non-treated	596	<.0001	3.41 (2.42-4.81)	301	<.0001	3.53 (1.96-6.35)
Entire cohort	3727	<.0001	4.74 (4.12-5.46)	2994	<.0001	4.87 (4.07-5.83)

Translation into clinical practice

For illustrative purposes, the preceding findings were translated into a practical example (**Figure 8**) to show the association of a composite surrogate endpoint (bilirubin level <1 time the ULN and alkaline phosphatase level less than the threshold) on 5-year transplant-free survival in different settings. Three groups of high-risk patients with PBC diagnosed after 1990 and treated with UDCA were defined at 2 different time points: baseline (upper panels) and after 1 year of UDCA therapy (lower panels). The subgroups were defined as follows: (1) all patients with PBC, (2) patients meeting the inclusion criteria of a recent clinical trial: bilirubin levels <2 times the ULN and either alkaline phosphatase levels >1.67 times the ULN or bilirubin levels >1 time the ULN,[24] and (3) patients with a bilirubin levels <3 times the ULN and either alkaline phosphatase levels >2 times the ULN or bilirubin levels >1 time the ULN. The surrogate endpoint was determined 1 year after inclusion. **Figure 8** shows the proportion of patients reaching the surrogate endpoint (left panels) and accompanying transplant-free survival (right panels).

If a bilirubin level <1 times the ULN and alkaline phosphatase level <2 times the ULN is used as a surrogate endpoint in high-risk PBC population 3 (light gray) and if patients are already treated with UDCA for 1 year (lower panels), the proportion of patients reaching the surrogate endpoint after an additional year of UDCA therapy is 18% (lower left panel), with an accompanying 5-year transplantation-free survival rate of 92% (lower right panel). The 5-year transplantation-free survival rate for patients not reaching the surrogate endpoint was 75%.

In summary, using higher alkaline phosphatase thresholds resulted in a lower proportion of patients not reaching the surrogate endpoint, with a poorer corresponding 5-year transplant-free survival. The 5-year transplant-free survival after continued UDCA therapy is irrespective of the chosen alkaline phosphatase threshold and risk population.

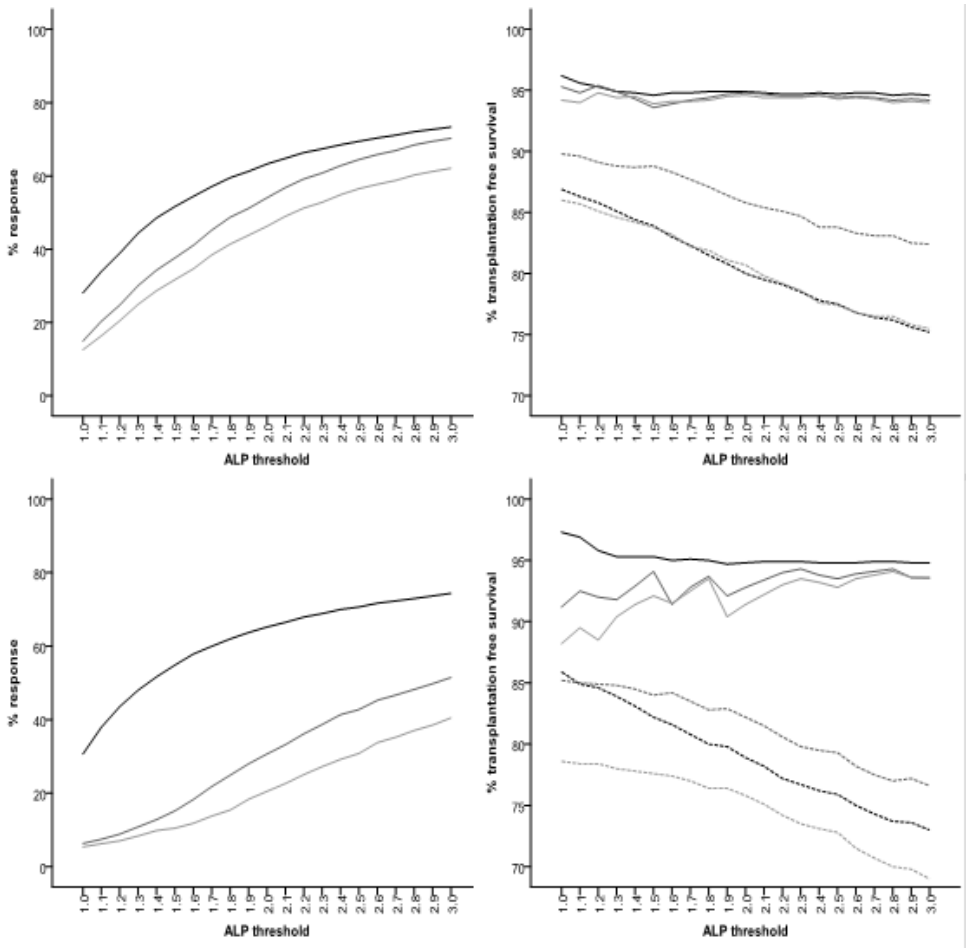

Figure 8. Translation into clinical practice

The association of a surrogate endpoint, defined as alkaline phosphatase <threshold and bilirubin <1xULN, on the 5-year liver transplantation-free survival in different settings. Inclusion (diagnosed >1990 and initiated on UDCA) was made at baseline (upper panels) and after one year on UDCA treatment (lower panels). Three high risk groups were defined as follows: 1) all patients (black lines), 2) bilirubin <2xULN and (alkaline phosphatase >1.67xULN or bilirubin >1xULN)[35] (dark grey lines), and 3) bilirubin <3xULN and (alkaline phosphatase >2xULN or bilirubin >1xULN) (light grey lines). The full lines represent the patients who reached the surrogate endpoint and the dotted lines those who did not. The left panels show the proportion of patients reaching the surrogate endpoint 1 year after inclusion and the right panels the corresponding 5-year transplant-free survival.

DISCUSSION

This study reports a robust and uniquely powered, independent evaluation of the largest meta-analysis of individual data on PBC to date. We unequivocally show that both increased serum alkaline phosphatase and bilirubin levels are strongly associated with reduced transplant-free survival in patients with PBC and that a combination of both variables improves prognostic prediction for patients. These associations are independent of use of UDCA and follow-up time and held for multiple subgroups. These data support that both alkaline phosphatase and bilirubin provide meaningful surrogate endpoints in PBC that can reasonably be used in clinical trials.

Prior studies have shown an association between normalization, percentage decreases or absolute decreases of alkaline phosphatase levels and improved prognosis with UDCA therapy.[4, 5, 7, 8, 18, 19] The present study reports for the first time a near log-linear association between alkaline phosphatase levels and transplant-free survival and clearly shows that the lower the alkaline phosphatase levels, the greater the transplant-free survival time. This applied not only to alkaline phosphatase levels during follow-up but also to baseline levels irrespective of subsequent UDCA therapy. The suitability of alkaline phosphatase as a surrogate endpoint for clinical benefit is further supported by the finding that the prognostic information provided by alkaline phosphatase levels was confirmed across a wide range of subgroups such as non-treated patients, relatively young patients, and patients with histologically early and late disease. This finding is of considerable clinical significance because alkaline phosphatase constitutes one of the 3 potential diagnostic criteria and is used routinely to assess disease activity.

Our study additionally confirms that as baseline bilirubin levels or bilirubin levels increase over time, the likelihood of survival correspondingly decreases.[10] The predictive ability of alkaline phosphatase levels was shown in addition to bilirubin to discriminate high-risk and low-risk patients. This is an important observation because bilirubin on its own is unsuitable as a surrogate endpoint in clinical trials because it is typically elevated only when the disease has progressed to the stage at which liver function becomes impaired. Most patients likely to be included in such studies will have normal levels precluding the possibility of observing potential beneficial treatment effects based on bilirubin alone.

It has been suggested that the best way to evaluate the utility of a biomarker as a good surrogate endpoint may be a meta-analysis of clinical trials of one or more interventions.[25] A 4-level hierarchy of evidence to consider the validation of surrogate endpoints has been proposed: Level 1: a true clinical-efficacy measure; Level 2: a validated surrogate endpoint (for a specific disease setting and class of interventions); Level 3: a non-validated surrogate endpoint, yet one established to be “reasonably likely to predict clinical benefit” (for a specific disease setting and class of interventions); Level 4: a correlate that is a measure

of biological activity but that has not been established to be a higher level.[26] The particular challenge of confirming biomarkers as surrogate endpoints in PBC is that there is only one approved treatment, and previous meta-analyses of published clinical trials that have been conducted in PBC have been interpreted in conflicting ways.[27-29] Interpretation of the data is compromised due to design issues, such as a lack of consistent long-term follow-up.[29, 30] Our approach was therefore to conduct a more rigorous patient level meta-analysis of existing cohorts of patients at centers across North America and Europe with long-term follow-up data of large numbers of patients with PBC. This design has sufficient power to intensively study alkaline phosphatase and bilirubin as potential surrogate endpoints in different settings, subpopulations, and time points. Based on these current results, we postulate that alkaline phosphatase and bilirubin levels are "reasonably likely to predict clinical benefit" in PBC.[26] This is of relevance to future trial design for new therapeutic agents.

Alternative surrogates have been suggested, such as liver histology,[31] which may provide key information on treatment effects in PBC. However, liver biopsy is not routinely conducted in patients with PBC. Given its invasive nature and small but well-recognized risks,[32] liver histology, with its added inherent sampling variability, is not an ideal surrogate for widespread use in patients with PBC. Non-invasive elastography-based assessment of liver fibrosis may potentially be used as a reliable alternative in the prediction of fibrosis[33]; however, further long-term evaluation is required in PBC. Similarly other biochemical surrogates have been suggested[5, 6, 34] but as of yet are not widely studied. We focused on the routine biochemical measurements that have been used for many years in both the diagnosis and management of patients with PBC, because this approach is likely to be the most easily applied in practice.

There are some limitations to our study. The availability of some clinical data (such as ascites, edema, pruritus, fatigue, or use of diuretics) and laboratory data (including prothrombin time, immunoglobulin M, and immunoglobulin G levels) in the individual databases varied considerably. In many cases, in particular when databases contained data of patients entered more than 10 to 20 years ago, it was not possible to collect these data consistently in a reliable way. Further, no uniform or generally accepted or validated methods had been used in the contributing centers to quantify subjective signs and symptoms. As a consequence, within the context of this study, we were unfortunately unable to include this type of information in our analyses and, in particular, were not able to calculate the Mayo risk score[16] and to compare the prognostic information provided by this established prediction tool with that provided by alkaline phosphatase and bilirubin.

Due to the nature of our study, biochemical data were not always available at the fixed time points during follow-up. This was mainly encountered when the original data had been collected more than 20 years ago. Data on dose changes or interruption of UDCA therapy was also not uniformly available. However, we believe that these limitations had no

major impact on the reliability of the results, considering the unique large size of the study population, the prospective nature of most of the data, the inclusion of both UDCA-treated and non-treated patients, the substantial incidence of clinical endpoints, and the duration of follow-up. Additionally, adjusting for missing data by multiple imputations of the data, the results did not change (**Table 7**).

Table 7. Multivariate analysis of treated and non-treated patients following multiple imputation to correct for missing data values

	Alkaline phosphatase >2.0xULN			**Bilirubin >1.0xULN**		
Cohorts	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Entire cohort	2.46	2.16-2.80	<.0001	4.80	4.13-5.57	<.0001
UDCA treated patients	2.49	2.15-2.88	<.0001	4.95	4.21-5.83	<.0001
UDCA non-treated patients	2.07	1.45-2.95	<.0001	3.79	2.58-5.59	<.0001

Based on our present results, any decrease in alkaline phosphatase or bilirubin levels translates into improved prognosis; lower levels are clearly associated with better transplant-free survival. In our population, the most discriminative alkaline phosphatase threshold after 1 year of follow-up was 2.0 times the ULN, which is an earlier proposed threshold,[18] although an alkaline phosphatase threshold of 1.5 times the ULN,[8] 1.67 times the ULN,[7, 19] or 3.0 times ULN would all work well as a surrogate endpoint in a clinical trial setting. For bilirubin, the choice of threshold is even clearer; the spline plots (**Figure 2**) suggest that a choice of bilirubin <1.0 times the ULN is reasonable. However, designing clinical trials implies the a priori requirement to estimate the quantitative effect of an experimental intervention on a given endpoint. Based on the current study, we are not able to translate these data into a specific threshold for a clinical trial in general.

In conclusion, our study shows that alkaline phosphatase and bilirubin levels strongly correlate with the ultimate outcomes of death and liver transplantation in patients with PBC; the lower the alkaline phosphatase and bilirubin levels the better the transplant-free survival times. This robust analysis suggests that these variables can reasonably be regarded as useful surrogate endpoints in clinical trials. There is a high unmet medical need for new therapies for this rare autoimmune liver disease, and this study provides an important impetus for the selection of appropriate endpoints and to facilitate the conduct of meaningful therapeutic intervention trials in the absence of long-term outcome studies.

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CHAPTER 2.2

Reply: Are levels of alkaline phosphatase and bilirubin surrogate markers of outcomes of patients with primary biliary cirrhosis?

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Gastroenterology 2015; 148: 860-861.

Dear Editor,

The comments of Giljaca, Stimac, and Gluud regarding our study are very much appreciated and timely. Patients with primary biliary cirrhosis (PBC) presently have only 1 therapy, ursodeoxycholic acid (UDCA), that is recommended universally for use globally; however, it fails to benefit all patients equally.

We agree that bilirubin and alkaline phosphatase have not been validated fully as true surrogate endpoints. In bringing together the Global PBC Study Group and publishing what we believe to be the largest individual patient data analysis to date in PBC, we comprehensively discussed the 4-level hierarchy of evidence for the validity of surrogate endpoints proposed by Fleming et al[1] as they relate to our findings. This hierarchy is highly comparable with the three-level approach of Bucher et al.[2] We acknowledge that, based on our data, bilirubin and alkaline phosphatase cannot be regarded as surrogate endpoints with the highest levels of evidence (level 1, true clinical- efficacy measure; level 2, validated surrogate endpoint). However, we clearly showed that the strong association between increased serum alkaline phosphatase and bilirubin levels and reduced transplant-free survival were independent of UDCA treatment and follow-up time and remained present for a range of subgroups. Given the comments raised, we have sought additional supportive data by analyzing alkaline phosphatase and bilirubin as time-dependent variables. UDCA-treated patients who continued to have alkaline phosphatase levels <2-fold the upper limit of normal (ULN) and bilirubin levels <1-fold the ULN throughout total follow-up had excellent 3-, 5- and 10-year survival rates (97%, 95%, and 91% respectively), compared with those whose liver biochemistry did not stay normal throughout follow-up (83%, 78%, and 62%, respectively, from onset of abnormal liver values) (**Figure 1A**). The same observation applies importantly for non-treated PBC patients. Those with alkaline phosphatase levels <2-fold the ULN and bilirubin levels <1-fold the ULN throughout follow-up had better 3-, 5-, and 10-year survival rates (92%, 89%, and 84%, respectively), than those without (74%, 61%, and 43%, respectively) (**Figure 1B**). Therefore, we continue to believe that our data robustly support the conclusion that both variables meet the criteria for level 3 evidence: variables "reasonably likely to predict clinical benefit."

Giljaca et al will agree that studies seeking to evaluate classic clinical outcomes, such as transplant-free survival, are unlikely to ever be feasible in PBC, and that there is a pressing need for alternative outcome measures. Additional validation studies of bilirubin and alkaline phosphatase as valid surrogates of outcome are necessary, as well as studies of other potential surrogate endpoints.

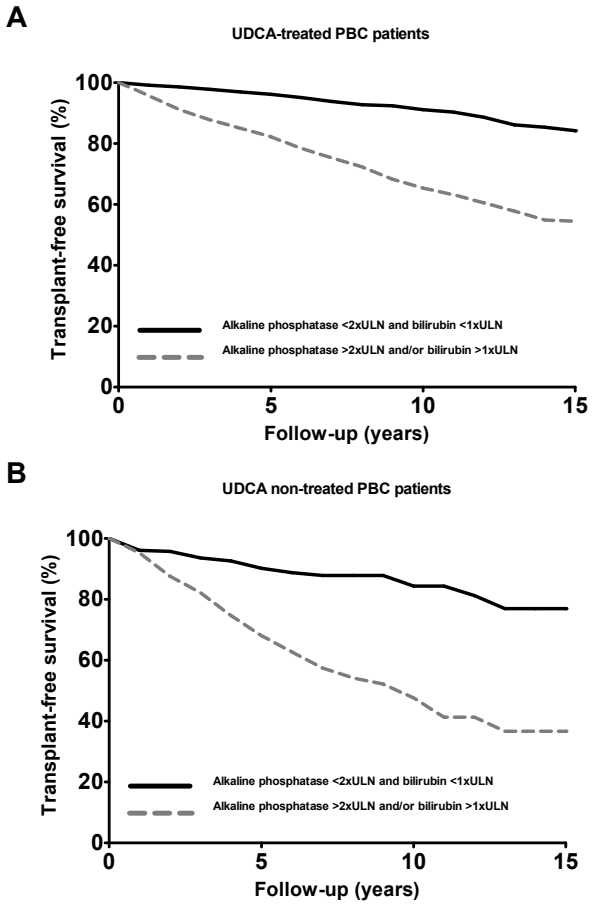

Figure 1. Liver transplant-free survival of primary biliary cirrhosis patients with and without alkaline phosphatase levels ≤2.0xULN and bilirubin levels ≤1.0xULN during follow-up
The survival curves were plotted using a clock-reset approach; patients who developed alkaline phosphatase levels >2.0xULN and/or bilirubin levels >1.0xULN during follow-up were censored in the group with alkaline phosphatase levels ≤2.0xULN and bilirubin levels ≤1.0xULN, and switched to the group with alkaline phosphatase levels >2.0xULN and/or bilirubin levels >1.0xULN. This analysis was performed in A) UDCA-treated and B) non-treated PBC patients.

Such studies as presented by the Global PBC Study Group are thus of critical importance for our ability to evaluate the efficacy of promising new therapies in PBC[3] as well as managing individual patient risk across the course of their disease.[4] Future approaches and efforts, however, need to be realistic and deliverable, so that timely progress can be made to address an overt and pressing unmet patient need for new treatments in PBC.

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CHAPTER 3

How the concept of biochemical response influenced the management of primary biliary cirrhosis in 831 patients over three decades

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ABSTRACT

Background & Aims: Criteria assessing biochemical response to ursodeoxycholic acid (UDCA) are established risk stratification tools in primary biliary cirrhosis (PBC). We aimed to evaluate to what extent biochemical response influenced patient management during a three decade period, and whether this changed over time.

Methods: 851 Dutch PBC patients diagnosed between 1988 and 2012 were reviewed to retrospectively assess patient management in relation to biochemical liver tests after one year UDCA treatment. Biochemical response was defined by the Paris-1 criteria.

Results: Response was assessable for 687/851 (81%) patients; 157/687 non-responders. During a follow-up of 8.8 years (IQR, 4.8-13.9) 141 died and 30 underwent liver transplantation. Transplant-free survival of non-responders (60%) was significantly worse compared with responders (87%) (p<.0001).

Management was modified in 46/157 (29%) non-responders. The most frequent change observed, noted in 26/46 patients, was an UDCA dosage increase. Subsequently, 9/26 (35%) non-responders became responder within the next 2 years. Steroid treatment was started in 1 patient; 19 patients were referred to a tertiary center. No trend towards more frequent management change over time was observed (p=0.10).

Conclusions: Changes in medical management occurred in a minority of non-responders. This can largely be explained by the lack of accepted response criteria and of established second-line treatments for PBC. Nevertheless, the observation that response guided management did not increase over time suggests that awareness of the concept of biochemical response requires further attention, particularly since new treatment options in PBC will becoming available soon.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease of autoimmune origin that mainly affects middle aged women.[1] To date, ursodeoxycholic acid (UDCA) at a recommended dosage of 13-15 mg/kg/day is the only approved therapy.[2, 3]

An association between biochemical variables and outcome during treatment with UDCA was first reported in 1999.[4] Angulo and colleagues showed that patients with serum alkaline phosphatase levels <2 times the upper limit of normal (ULN) following 6 months of UDCA treatment were less likely to have treatment failure. Subsequent studies found that not only levels of alkaline phosphatase,[5, 6] but also other biochemical variables including aspartate transaminase (AST), bilirubin and albumin values following one or two years of UDCA treatment were predictive of liver transplant-free survival.[7-10] Generally, patients fulfilling criteria for biochemical response were shown to have a normal life expectancy, comparable with a matched general population, while non-responders remained at risk for requiring liver transplantation or premature death.[5]

Despite the clear relevance of biochemical response to UDCA it has not been established whether biochemical response is considered an important objective in clinical practice and is used to guide further decision-making, in particular on possible additional second-line treatment. Therefore we aimed to assess the impact of biochemical response to UDCA on management decisions in a large and nationwide cohort of PBC patients.

PATIENTS AND METHODS

Patient population

Patients were derived from a Dutch multicenter study[11] and a large epidemiological study regarding primary sclerosing cholangitis and primary biliary cirrhosis in the Netherlands.[12] The protocol for this project was approved by the central Committee for Research Ethics in Utrecht and the local ethics committees of participating hospitals (trialregister.nl no.: NTR2813).

Patients were diagnosed according to established criteria,[2, 3] and included between November 1988 and December 2011 across 43 university and general hospitals. Patients with concomitant liver disorders, such as viral, alcoholic, and autoimmune hepatitis were excluded.

Endpoints

For current study, entry (baseline) was defined as start date of UDCA therapy. Biochemical response to UDCA treatment was retrospectively assessed according to Paris-1 criteria,[7] generally accepted as the criteria with best performance in predicting outcome.[13, 14] Paris-1 was defined as alkaline phosphatase <3 times the ULN, AST <2 times the ULN and bilirubin ≤1 mg/dl after one year of UDCA treatment, and Paris-2 criteria,[9] defined as alkaline phosphatase ≤1.5 times the ULN, AST ≤1.5 times the ULN and bilirubin ≤1 mg/dl after one year of UDCA treatment. A composite of liver transplantation and death was used as clinical endpoint. Patients who did not reach a clinical endpoint were censored at their last follow-up visit.

Data collection

The original database comprised clinical and laboratory data at baseline and during follow-up. Clinical data included gender, age, details about the diagnosis of PBC, antimitochondrial antibody (AMA) status, liver histology obtained within one year of study entry, UDCA treatment (start date and dosage), and outcome (liver transplantation and death). Laboratory data (alkaline phosphatase, AST, alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GT), bilirubin, albumin and platelets) were yearly collected.

During site visits additional follow-up information was gathered from medical charts for UDCA non-responders within the next two years following the (retrospective) assessment of biochemical response (**Figure 1**). Data collected included changes in UDCA dosage, prescription of additional medication, and referral to tertiary centers.

Figure 1. Study timeline

The study started at initiation of ursodeoxycholic acid (UDCA). Biochemical response to UDCA was retrospectively calculated after one year therapy using Paris-1 criteria.[7] Subsequently, modifications in treatment management were evaluated in the following two years.

Statistical analyses

Normally distributed data was expressed as mean ± standard deviation and skewed data was expressed as median and interquartile range (IQR). Differences between responders and non-responders were assessed by using the independent t-test and non-parametric Mann-Whitney U test, respectively. To assess differences between responders and non-responders concerning categorical variables the Pearson's chi-squared test was used. Kaplan-Meier method was applied for time-to-event analysis and survival difference was tested

with log-rank test. Logistic regression modelling was performed to assess the association between baseline factors and UDCA response after one year of follow-up in univariate and multivariable approaches.

A p<.05 was considered statistically significant. Analyses were performed using statistical package of IBM SPSS Statistics 21.0 (SPSS Inc., Chicago IL, USA).

RESULTS

Study cohort

The study cohort comprised 851 UDCA-treated PBC patients. The Paris-1 criteria could be assessed in 687 (81%) patients; 77% of patients were classified as responder and 23% as non-responder (**Figure 2**). Non-responders were generally younger, at a more advanced disease stage, diagnosed in an earlier era and had higher serum bilirubin, alkaline phosphatase, AST and ALT values and lower albumin at baseline (**Table 1**).

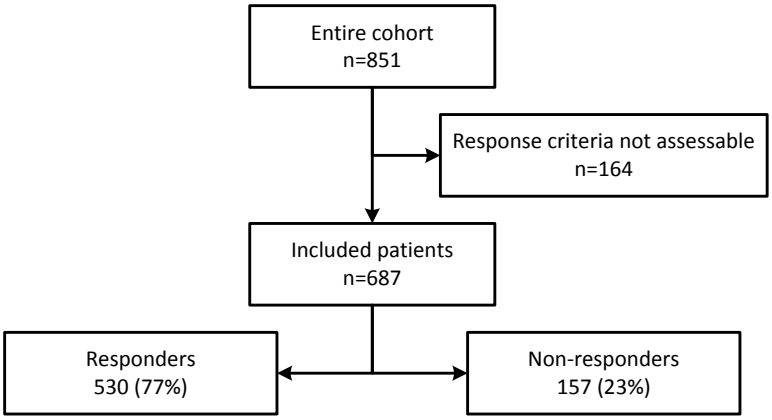


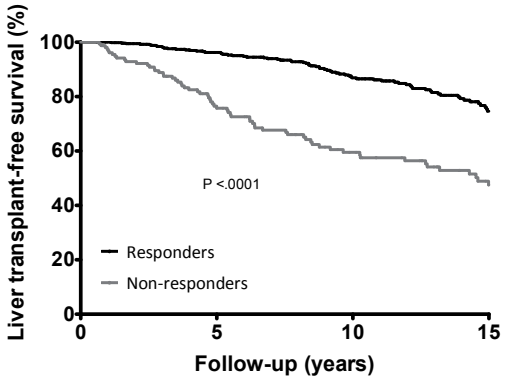
Figure 2. Flowchart
Flow diagram of patients included in this study.

Table 1. Clinical and biochemical characteristics at baseline of responders and non-responders according to Paris-1 criteria

	Responders N=530	Non-responders N=157	P-value
Mean age at study entry, y	57.1±11.6	53.7±12.9	.002
Female, n (%)	461 (87%)	136 (87%)	.91
AMA+, n (%)[a]	501 (95%)	143 (91%)	.22
Biochemical disease stage,[b] n (%)			<.0001
Early	306 (58%)	42 (27%)	
Moderately advanced	63 (12%)	54 (34%)	
Advanced	5 (1%)	29 (19%)	
Not available	156 (29%)	32 (20%)	
Median year of diagnosis (IQR)	2000 (1993-2005)	1995 (1988-2002)	<.0001
Year of diagnosis, time frame	1973-2011	1961-2011	
Median UDCA dosage/kg[c]			
Year of diagnosis <2000	9.84 (9.04-11.36)	9.49 (8.70-10.38)	.056
Year of diagnosis ≥2000	13.38 (11.25-15.00)	13.43 (10.81-16.19)	.46
Laboratory data at entry			
Bilirubin (xULN)	0.57 (0.42-0.76)	1.17 (0.67-2.03)	<.0001
Not available	42 (8%)	12 (8%)	
Alkaline phosphatase (xULN)	1.95 (1.32-3.05)	4.15 (2.56-6.19)	<.0001
Not available	25 (5%)	5 (3%)	
Aspartate transaminase (xULN)	1.33 (0.95-2.00)	2.20 (1.53-3.10)	<.0001
Not available	23 (4%)	7 (4%)	
Alanine aminotransferase (xULN)	1.52 (1.02-2.50)	2.49 (1.54-3.65)	.0053
Not available	20 (4%)	5 (3%)	
Albumin (xLLN)	1.14 (1.06-1.22)	1.08 (0.94-1.20)	<.0001
Not available	148 (28%)	31 (20%)	

[a]AMA status was not available for 1 patient (responder)
[b]Disease severity was classified according to bilirubin and albumin levels. Early disease, normal albumin and bilirubin; moderately advanced disease, abnormal albumin or bilirubin; advanced disease, both albumin and bilirubin abnormal.[11]
[c]dosage/kg was not calculable for 90/687 (13%) patients: 71/530 (13%) responders and 19/157 (12%) non-responders.

The median follow-up period of the entire cohort was 8.8 years (IQR, 4.8-13.9) and follow-up for responders was significantly longer than for non-responders (9.2 versus 7.8 years respectively, p=.047). During follow-up 141 patients died and 30 underwent a liver transplantation (47 and 24 non-responders, respectively). Ten-year transplant-free survival for non-responders was significantly lower than for responders (60% versus 87%, p<.0001) (**Figure 3**).



Responders	530	402	242	104	*Patients at risk*
Non-responders	157	98	60	35	

Figure 3. Liver transplant-free survival rates according to biochemical response (Paris-1 criteria)
Liver transplant-free survival estimated with Kaplan Meier. The 10-year transplant-free survival of non-responders was significantly lower than of responders (60% versus 87%, P <.0001).

Modification in management

Management was modified in 46/157 (29%) non-responders. The most frequently applied change was an increase of the UDCA dosage (26/46, 57%). Steroid therapy was started in only one non-responder. No other drugs as second-line therapy were prescribed. Nineteen patients were referred for second opinion to a tertiary center. For 6/157 (4%) non-responders management changes were not extractable from medical charts.

The relation between publications on biochemical response and changes in patient management

In 1999 a first study was published addressing the significance of biochemical response.[4] In our cohort the therapeutic approach was modified in 33/104 (32%) of the non-responders before 1999 as compared with 13/53 (25%) after that year (p=.10). The key paper of Pares et al. on biochemical response was published in 2006.[5] When comparing the proportion of management changes in non-responders before and after 2006, again no clear difference was found (p=.62).

Impact of UDCA dosage increase

After one year of treatment with UDCA the dosage was increased in a number of non-responders and responders within the following two years. Importantly, 9/26 (35%) of the non-responders became responder within the next two years following dosage increase**.** When applying the more strict Paris-2 criteria for response,[9] 6/48 (13%) of non-responders became responder.

Eighteen of twenty-six (69%) non-responders in whom the UDCA dosage was increased were initially dosed inadequately (median daily dosage 9.60 mg/kg; IQR, 8.80-11.16 mg/kg) according to current treatment guidelines.[2, 3] Further analysis showed that this mainly applied to patients diagnosed in 1999 or before (median dosage 9.33 mg/kg; IQR, 8.21-9.93 mg/kg) and not for those diagnosed in 2000 or thereafter (median dosage 12.69 mg/kg; IQR, 10.92-17.98 mg/kg).

Baseline factors predictive of response

Since biochemical response is of major importance in risk stratification baseline factors were determined predictive of response (**Table 2**). Higher serum albumin levels and higher UDCA dosage per kg were associated with increased response to UDCA treatment, whereas higher levels of bilirubin, alkaline phosphatase, AST, ALT and more advanced disease stage (defined as abnormal bilirubin and/or albumin[11]) were all associated with decreased response. On multivariable analysis more advanced disease stage, lower UDCA dosage per kg and higher serum alkaline phosphatase levels were independent factors predictive of poor response.

Table 2. Baseline factors predictive of response according to Paris-1 criteria

	Univariate analysis		Multivariable analysis	
	Odds ratio (95% CI)	**P-value**	**Odds ratio (95% CI)**	**P-value**
Male sex	1.03 (0.61-1.74)	ns	-	-
AMA positivity	1.75 (0.90-3.42)	ns	-	-
Advancing age at study entry	1.02 (1.01-1.04)	.0022	1.04 (1.01-1.06)	.0021
Year of diagnosis	1.06 (1.03-1.08)	<.0001	-	-
UDCA dosage per kg	1.09 (1.02-1.16)	.0095	1.15 (1.05-1.26)	.0025
Disease stage[a]				
Moderate	0.16 (0.10-0.26)	<.0001	0.18 (0.10-0.32)	<.0001
Advanced	0.02 (0.01-0.06)	<.0001	0.02 (0.01-0.08)	<.0001
Bilirubin (xULN) values	0.41 (0.32-0.54)	<.0001	-	-
Alkaline phosphatase (xULN) values	0.65 (0.59-0.72)	<.0001	0.69 (0.60-0.78)	<.0001
AST (xULN) values	0.78 (0.69-0.89)	.00012	-	ns
ALT (xULN) values	0.90 (0.82-0.99)	.026	-	-
Albumin (xLLN) values	33.05 (7.86-138.93)	<.0001	-	-

[a]Disease stage based on albumin and bilirubin according to biochemical disease classification of ter Borg et al.[11]

DISCUSSION

To our knowledge, this large nationwide multi-center cohort study in PBC is the first to assess potential changes in patient management prompted by the 1-year response to UDCA treatment. We found that non-response to treatment did not result in management changes in nearly two-thirds of cases. In those patients in whom management was influenced, the most frequent change was an increase in the dosage of UDCA. Our data further show that the proportion of UDCA non-responders in whom management was modified did not increase over time, suggesting that awareness of the concept of biochemical response in clinical practice may still be suboptimal.

Few data are available with respect to treatment policy based upon objective response criteria in PBC. Recently, preliminary data of an online survey among 200 gastroenterologists and hepatologists in the UK were presented.[15] 42% of gastroenterologists and 76% of hepatologists stated they used biochemical response criteria (e.g. Paris-1 criteria[7] or Barcelona criteria[5]) to evaluate UDCA treatment. However, no information was provided about further treatment decisions based upon the observed response.

Obviously, the results of our study should be interpreted with caution. In particular, it must be recognized that the majority of included patients were treated with UDCA well before emergence of the concept of biochemical response and that, in the context of this study, this response was assessed retrospectively. Therefore, by definition, decisions with respect to patient management could not have been influenced by assessing treatment response with one of the currently available tools. Irrespective of a formal response evaluation, however, our data suggest that management was modified in only a minority of cases despite persistently, occasionally markedly, abnormal biochemical liver tests. Moreover, our data demonstrate that during the last decade, despite increased awareness of the importance of sufficient biochemical improvement upon treatment with UDCA, this did not translate yet into an increase in response-guided management in general medical practice.

Another major factor that must be stressed when interpreting the results of the present study is the lack of evidence-based alternative treatments for PBC until now. This may largely explain why potentially effective drugs, including budesonide and fibrates, were rarely used. During recent years, evidence is accumulating that fibrates may have an additional, beneficial effect in UDCA-treated PBC.[16-20] The same applies to budesonide[21-23] and obeticholic acid,[24] drugs that are currently undergoing randomized controlled trial evaluation. It seems likely that within a few years the therapeutic scenario in PBC will have changed considerably and an evidence-based approach of response guided treatment in PBC will be reality, potentially with a number of second-line treatment options available.

Our study emphasizes the importance of adequate UDCA dosage. About 40% of UDCA non-responders in whom the dosage was increased became responder according to the criteria we used. Indeed, a multivariable analysis of factors predictive of response confirmed that higher UDCA dosage per kilogram was an independent predictor of response. These findings are in line with previous studies showing that UDCA doses in the range of 13-15 mg/kg/day are more effective than lower doses.[25, 26] Therefore, adequate dosing of UDCA remains of crucial importance.

A potential weakness of our study is its retrospective character, occasionally necessitating the retrieval of data from hand-written patient records more than 20 years old. Also, it may be very well possible that management changes did occur more frequently than documented in the present study, but that this was after more prolonged follow-up. On the other hand, we believe that this study of a large PBC population gives unique and representative insight into general clinical practice since it was not restricted to high-volume university centers but also involved many smaller community hospitals.

In conclusion, in this long-term cohort study of PBC we found that changes in medical management occurred in a minority of patients who, retrospectively, responded insufficiently to UDCA treatment. During the last decade this did not change despite the emergence of established stratification tools. Now new therapeutic options for PBC are becoming available awareness of the concept of biochemical response requires further attention.

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CHAPTER 4

Development and validation of a scoring system to predict outcomes of patients with primary biliary cirrhosis receiving ursodeoxycholic acid therapy

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Gastroenterology 2015, epub ahead of print

ABSTRACT

Background & Aims: Approaches to risk stratification for patients with primary biliary cirrhosis (PBC) are limited, single-center based, and often dichotomous. We aimed to develop and validate a better model for determining prognoses of patients with PBC.

Methods: We performed an international, multicenter meta-analysis of 4119 patients with PBC treated with ursodeoxycholic acid (UDCA) at liver centers in 8 European and North American countries. Patients were randomly assigned to derivation (n=2488, 60%) and validation cohorts (n=1631, 40%). A risk score (GLOBE score) to predict transplantation-free survival was developed and validated with univariate and multivariable Cox regression analyses using clinical and biochemical variables obtained after 1 y UDCA therapy. Risk score outcomes were compared with the survival of age-, sex-, and calendar time-matched members of the general population. The prognostic ability of the GLOBE score was evaluated alongside those of the Barcelona, Paris-1, Rotterdam, Toronto, and Paris-2 criteria.

Results: Age (hazard ratio [HR], 1.05; 95% confidence interval [CI], 1.04–1.06; p<.0001); levels of bilirubin (HR, 2.56; 95% CI, 2.22–2.95; p<.0001), albumin (HR, 0.10; 95% CI, 0.05–0.24; p<.0001), and alkaline phosphatase (HR, 1.40; 95% CI, 1.18–1.67; p=.0002); and platelet count (HR/10 units decrease, 0.97; 95% CI, 0.96–0.99; p<.0001) were all independently associated with death or liver transplantation (C statistic derivation, 0.81; 95% CI, 0.79–0.83, and validation cohort, 0.82; 95% CI, 0.79–0.84). Patients with risk scores >0.30 had significantly shorter times of transplant-free survival than matched healthy individuals (p<.0001). The GLOBE score identified patients who would survive for 5 y and 10 y (responders) with positive predictive values of 98% and 88%, respectively. Up to 22% and 21% of events and non-events, respectively, 10 y after initiation of treatment were correctly reclassified in comparison with earlier proposed criteria. In subgroups of patients <45 y, 45–52 y, 52–58 y, 58–66 y, and ≥66 y old, age-specific GLOBE-score thresholds beyond which survival significantly deviated from matched healthy individuals were –0.52, 0.01, 0.60, 1.01 and 1.69, respectively. Transplant-free survival could still be accurately calculated by the GLOBE score with laboratory values collected at 2–5 y after treatment.

Conclusions: We developed and validated scoring system (the GLOBE score) to predict transplant-free survival of UDCA-treated patients with PBC. This score might be used to select strategies for treatment and care.

INTRODUCTION

Primary biliary cirrhosis (PBC) is the most common of the autoimmune liver diseases, with 1 in 1000 women over the age of 40 affected.[1] Prognosis largely depends on the development of liver cirrhosis and its complications.[2] Presently, treatment with ursodeoxycholic acid (UDCA) represents the global standard of care,[2, 3] and can delay histological progression[4-6] and can improve long-term survival.[7, 8] However, UDCA is not an uniformly effective drug and the prognosis of patients insufficiently responding to treatment is markedly worse compared with the general population.[9] Reliable identification of such individuals is of key importance to clinical management, particularly for selecting those who could benefit from additional second-line medical therapies, but equally for identification of patients at low risk of developing end-stage liver disease.

A number of existing stratification tools, using biochemical liver tests applied after one or two years of UDCA exposure, will readily identify patients with or without sufficient treatment response.[9-13] Paris-1 criteria is generally considered as the one with best predictability of transplant-free survival as validated in large studies, such as the UK-PBC consortium and our own group.[11, 14-16] However, Paris-1 and other criteria were all based on dichotomized variables, potentially leading to loss of important predictive information. And even more important there is a relatively high disagreement between the different criteria in classifying someone among low- and high risk groups.[17]

The Global PBC Study Group has representative data from an international PBC research collaboration that has already evaluated biochemical surrogates of disease progression and liver cancer risk.[16, 18] The aim of present study was to utilise our unique dataset, alongside representative healthy population data, to develop a new unifying score with optimal ability to identify UDCA-treated patients with an insufficient treatment effect, based on readily obtainable, biochemical and clinical variables.

METHODS

Study population and design

Patients were derived from the Global PBC Study Group database. This study group is an international and multicenter collaboration between 15 liver centers from 8 North American and European countries, which combined individual patient data from major long-term follow-up cohorts. Most cohorts included prospectively collected follow-up data. All patients had an established diagnosis of PBC[2, 3] and characteristics of the study population have been previously described elsewhere.[18] For the current study only those patients treated with UDCA were included. Patients were excluded if follow-up data were insufficient

or unavailable, the start date of treatment or the exact date of major clinical events was unknown or in case of concomitant liver disease. Collected clinical and laboratory data included gender, age, PBC diagnosis, liver histology, treatment (type of medication, dosage and duration), duration and last date of follow-up, baseline antimitochondrial antibody status, baseline and yearly laboratory values (serum alkaline phosphatase, total bilirubin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and platelets and outcomes (death and cause of death, liver transplantation, hepatocellular carcinoma, ascites and variceal bleeding).

Ethical approval

This study was conducted in accordance with the protocol and the principles of the Declaration of Helsinki. The protocol was approved by the Institutional Research Board of the corresponding center, and at each participating center, in accordance with local regulations.

Statistical analysis

The study population was divided into a two cohorts, a derivation series comprising a randomly selected group of 2488 patients (60%), with the remainder serving as of a validation cohort (n=1631, 40%). Follow-up commenced at the start of UDCA therapy. Clinical outcome consisted of a composite endpoint of liver transplantation and all-cause mortality with the first event considered. Patients failing to reach a clinical endpoint were censored at time of last follow-up.

For development of our risk score only easily and readily available clinical and laboratory variables were considered: sex, baseline age, and serum bilirubin, alkaline phosphatase, AST, ALT, albumin, platelet count, AST/ALT ratio, and AST to platelet ratio index (APRI) at one year follow-up. Where indicated, continuous variables underwent natural logarithmic transformation to correct for non-linearity. Multiple imputation was also applied to account for missing data wherein ten complete datasets were constructed by imputing missing values (SAS Proc MI, MCMC method; SAS 9.3).[19]

Time-to-event analysis was conducted using univariate and multivariable cox proportional hazard regression, and a final model was selected by comparing the goodness of fit criteria (Akaike Information Criteria and maximum-likelihood estimation). The final model was checked for potential confounding factors and interactions between the included variables. A penalised maximum likelihood estimation was used to account for over fitting of the model.[20 21]

A prognostic index (GLOBE score) was calculated with the beta coefficients of variables included in the final penalized multivariable model, along with a baseline survival estimate $S_0(t)$, t=time. The GLOBE score was centered on the median in the derivation set.

The overall discriminative ability of the GLOBE score was measured with C statistic in both the derivation and validation cohort. To visualise the discriminate ability Kaplan-Meier curves were plotted of 5 risk groups according to the 10th, 40th, 60th and 90th percentiles of the GLOBE score.

Calibration of the GLOBE score was tested within the validation set.[22] The calibration slope was calculated by estimating the regression coefficient on the GLOBE score. The necessity of recalibration was further tested by performing a Cox regression analysis on the variables included in the final model and including the GLOBE score with the regression coefficient constrained to 1. A good model fit was reached when the joint test of all beta coefficients did not significantly differ from 0. The accuracy of the baseline survival estimate $S_0(t)$ was investigated by comparing the predicted survival probabilities of the 5 risk groups as defined above in the validation set with the observed Kaplan-Meier survival probabilities.

In order to identify patients in whom prognosis significantly deviates from normal, the score was calculated beyond which prognosis was significantly worse than of a normal population. To determine this threshold, survival of patients with GLOBE scores below the tenth percentile was compared with that of an age-, sex- and calendar time matched Dutch population. During subsequent steps patients with scores within the next ten percentiles were added to the population and calculations were repeated until survival significantly deviated from that of the matched normal population (non-responders). Data of the matched population, a population with a life-expectancy comparable with that of the other participating countries, were retrieved from a Dutch registry (Statistics Netherlands, www.cbs.nl). The performance of the GLOBE score using this threshold was assessed with sensitivity, specificity, negative predictive value and positive predictive value at 5- and 10-year follow-up. For this purpose a GLOBE score below the aforementioned threshold was considered as a positive test and the absence of adverse outcome was considered as an event.

The overall predictive performance of previously reported tools (the Barcelona,[9] Paris-1,[10] Rotterdam,[11] Toronto[12] and Paris-2 criteria[13]) was assessed with C statistic. To quantify the improvement in discriminative ability the net reclassification improvement (NRI) for both events and non-events[23, 24] during the first 5 and 10 years follow-up was calculated.

All analyses were 2 sided. $p<.05$ was considered statistically significant if not otherwise specified. Statistical analyses were performed with IBM SPSS Statistics 22.0 (SPSS Inc, Chicago, IL, USA) and SAS 9.3 (SAS institute, Cary, NC, USA).

RESULTS

Clinical characteristics of the derivation cohort

The derivation cohort consisted of 2488 subjects with PBC, with a median age of 54.6 years at the time of diagnosis (**Table 1**). During a median follow-up of 7.8 years (interquartile range (IQR) 4.0-12.1) 558 patients reached a clinical endpoint; 369 patients died and 189 patients underwent liver transplantation (center specific characteristics are described in **Table 2**). The 5-, 10- and 15-year transplant-free survival rates were 90.0%, 77.5% and 65.6% respectively, as shown in **Figure 1**.

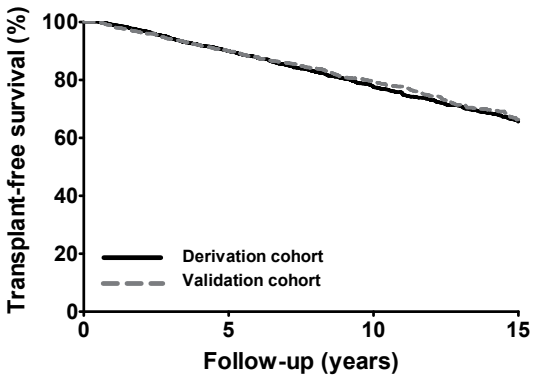
Table 1. Baseline characteristics

	Derivation cohort (n=2488)	Validation cohort (n=1631)
Age, years, mean (SD)	54.6 (11.7)	54.8 (11.9)
Female, n (%)	2253 (90.6%)	1453 (89.1%)
AMA+, n (%)	2208 (88.7%)	1425 (87.4%)
Year of diagnosis	1997 (1991-2003)	1998 (1992-2004)
Year of diagnosis, time frame	1961-2012	1970-2012
Histological disease stage, n (%)[1]		
Stage I	336 (27.9%)	237 (28.6%)
Stage II	337 (28.0%)	211 (25.5%)
Stage III	171 (14.2%)	125 (15.1%)
Stage IV	138 (11.5%)	87 (10.5%)
Not available	222 (18.4%)	167 (20.2%)
Serum bilirubin (xULN)	0.65 (0.45-1.00)	0.67 (0.45-1.05)
Serum alkaline phosphatase (xULN)	2.11 (1.37-3.79)	2.16 (1.33-3.78)
Serum AST (xULN)	1.46 (0.94-2.20)	1.45 (0.94-2.27)
Serum ALT (xULN)	1.68 (1.05-2.59)	1.63 (1.00-2.67)
Serum albumin (xLLN)	1.14 (0.15)	1.14 (0.17)
Platelet count	246 (90)	240 (96)
AST/ALT ratio	0.90 (0.72-1.16)	0.92 (0.73-1.18)
APRI	0.60 (0.34-1.01)	0.62 (0.36-1.09)
Laboratory data after one year[2]		
Serum bilirubin (xULN)	0.57 (0.41-0.86)	0.59 (0.41-0.90)
Serum alkaline phosphatase (xULN)	1.34 (0.93-2.26)	1.36 (0.93-2.25)
Serum AST (xULN)	0.90 (0.67-1.40)	0.90 (0.67-1.42)
Serum ALT (xULN)	0.90 (0.60-1.53)	0.90 (0.59-1.47)
Serum albumin (xLLN)	1.14 (0.15)	1.14 (0.17)
Plateletcount	237 (90)	237 (96)
AST/ALT ratio	1.03 (0.79-1.33)	1.03 (0.81-1.33)
APRI	0.38 (0.25-0.66)	0.39 (0.26-0.72)

[1]Baseline biopsies (obtained within one year of start of UDCA) were available in 1204/2488 (48%) patients of the derivation cohort and in 827/1631 (51%) patients of the validation cohort.
[2]laboratory variables are expressed as median and interquartile range; serum albumin and platelet count are expressed as mean and standard deviation.

Table 2. Center specific characteristics of the study population

	Derivation cohort							Validation cohort						
		Year of diagnosis		Follow-up (years)		Endpoints			Year of diagnosis		Follow-up (years)		Endpoints	
	N	*Median (IQR)*		*Median (IQR)*		*Death*	*LTx*	*N*	*Median (IQR)*		*Median (IQR)*		*Death*	*LTx*
USA, (*Rochester*)	349	2000	1997-2006	4.9	2.6-10.1	70	30	241	2000	1997-2007	4.1	2.1-9.7	32	36
The Netherlands, *(Nationwide cohort*)	515	1998	1992-2005	9.1	4.9-14.6	96	19	323	2000	1994-2006	8.5	4.5-13.1	57	12
Canada, (*Toronto*)	301	1999	1994-2003	7.6	4.4-11.4	24	15	228	1999	1995-2004	7.5	4.6-11.7	10	12
Italy, (*Padua*)	166	1997	1991-2005	8.0	4.3-14.3	40	2	110	2000	1995-2006	6.1	3.1-11.9	19	2
UK, (*Birmingham*)	175	2003	2000-2007	5.7	3.1-9.7	29	27	110	2003	2000-2007	6.8	4.2-10.0	21	14
French, (*Paris*)	221	1988	1986-1993	5.3	2.1-8.8	26	25	127	1987	1985-1992	6.2	2.1-9.2	12	15
USA, (*Dallas*)	191	1993	1990-1996	9.1	7.1-11.7	11	18	135	1993	1991-1996	8.5	6.4-11.5	4	14
Italy, (*Milan, 2 centers*)	232	1990	1984-1997	8.7	4.7-12.9	39	15	154	1989	1985-1994	8.2	5.0-13.5	29	6
Spain, (*Barcelona*)	156	1995	1991-2000	12.3	7.7-16.5	22	16	110	1996	1992-2000	12.2	8.1-16.3	9	7
Belgium, (*Leuven*)	95	2000	1992-2006	7.9	3.9-13.1	9	15	41	2004	1995-2009	5.3	2.6-11.1	2	4
UK, (*London*)	36	1994	1990-1999	9.0	4.8-13.7	1	4	20	1996	1991-2001	8.8	5.1-11.1	1	3
Canada, (*Edmonton*)	30	2004	2001-2006	5.9	4.9-8.3	2	3	23	2003	1995-2006	6.5	3.8-9.2	1	6
USA, (*Seattle*)	21	2008	2002-2010	2.7	1.6-9.5	0	0	9	2008	2006-2010	2.9	1.6-6.2	0	0
Total	2488	1997	1991-2003	7.8	4.0-12.1	369	189	1631	1998	1992-2004	7.5	3.8-11.8	197	131



Derivation cohort	2488	1688	886	345	*Patients at risk*
Validation cohort	1631	1091	567	202	

Figure 1. Liver transplant-free survival probability
Transplant-free survival probability of patients with primary biliary cirrhosis in the derivation cohort (N = 2488, solid line) and the validation cohort (N = 1631,dotted line).

Construction of the GLOBE score

Following univariate Cox regression analyses older age at start of UDCA therapy, male sex, elevated serum bilirubin, alkaline phosphatase, AST and ALT levels, lower serum albumin levels and thrombocytopenia and higher AST/ALT and APRI ratios after one year of UDCA therapy were all associated with higher risk of liver transplantation or death (**Table 3**). The final penalized multivariable model comprised age, bilirubin, albumin, alkaline phosphatase and platelet count as independent predictors of liver transplantation or death (**Table 3**). No significant interactions were found between these variables (**Table 4).**

Table 3. Univariate and multivariable cox regression analysis for liver transplantation or death within the derivation cohort (n=2488)

	Univariate analyses			Multivariable analyses[1]		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**
Age at baseline, *per year*	1.038	1.030-1.046	<.0001	1.045	1.035-1.056	<.0001
Male sex	1.913	1.510-2.425	<.0001	-	-	-
Bilirubin xULN[2]	3.215	2.903-3.562	<.0001	2.560	2.219-2.952	<.0001
Alkaline phosphatase xULN[2]	1.929	1.687-2.204	<.0001	1.399	1.175-1.665	.0002
AST xULN[2]	2.560	2.220-2.952	<.0001	-	-	-
ALT xULN[2]	1.401	1.232-1.594	<.0001	-	-	-
Albumin xLLN	0.014	0.007-0.028	<.0001	0.104	0.045-0.238	<.0001
Platelet count (*10^9/L), *per 10 units*	0.993	0.992-0.995	<.0001	0.970	0.961-0.990	<.0001
AST/ALT ratio[2]	2.537	1.998-3.223	<.0001	-	-	-
APRI[2]	2.235	1.985-2.518	<.0001	-	-	-

Abbreviations: HR, hazard ratio; LLN, lower limit of normal; ULN, upper limit of normal; AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, AST to Platelet Ratio Index.
[1]A P-value of <0.01 was considered as statistically significant.
[2]These biochemical variables were transformed with natural logarithm.

Table 4. Interactions tested between individual variables of the GLOBE score

	Bilirubin	Albumin	Alkaline phosphatase	Platelet count
Age	0.94*	0.25*	0.97*	0.75*
Bilirubin	-	0.54*	0.63*	0.74*
Albumin	-	-	0.95*	0.89*
Alkaline phosphatase	-	-	-	0.03*

*P-values of interaction terms tested in the final multivariable Cox regression model; a P <.01 was considered statistically significant.

The GLOBE score was calculated as follows:

GLOBE score = 0.044378**age at start of UDCA therapy* + 0.93982*LN(*bilirubin times the upper limit of normal [ULN] at 1 year follow-up*) + 0.335648*LN(*alkaline phosphatase times the ULN at 1 year follow-up*) – 2.266708**albumin level times the lower limit of normal (LLN) at 1 year follow-up* – 0.002581**platelet count per* 10^9*/L at 1 year follow-up* + 1.216865.

The distribution of the GLOBE score is plotted in **Figure 2**. The baseline survival curve at the mean GLOBE score $S_0(t)$ was: 0.9652, 0.9385, 0.8429, 0.7361 at 3-, 5-, 10- and 15-year follow-up respectively. The survival S(t) for any given patients was then calculated by $S(t) = S_0(t)^{\exp(\text{GLOBE score})}$.

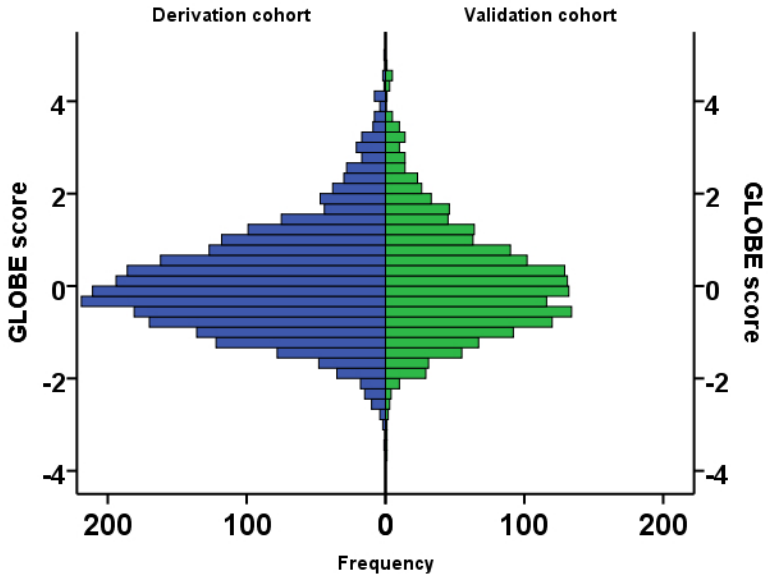


Figure 2. Distribution of the GLOBE score within the derivation and validation cohort

Example:
For a 50-year old patient with a bilirubin level of 1 time the ULN, an alkaline phosphatase level of 3 times the ULN, an albumin level of 1.5 time the LLN and a platelet count of 250 per 10^9/L:
GLOBE score = -0.24; transplant-free survival at 5-year, S(5) = 95.1% and at 10-year, S(10) = 87.4%.

The overall predictive ability of the GLOBE score for transplantation or death, calculated with C statistic, was 0.81 (95% CI, 0.79-0.83).

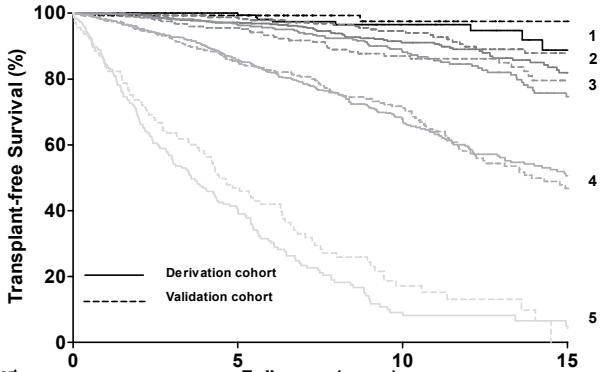
Validation of the GLOBE score
The clinical characteristics of the validation cohort (n=1631) are described in **Table 1**. During a median follow-up time of 7.5 years (IQR 3.8-11.8) 328 patients reached a clinical endpoint; 197 died and 131 received a liver transplant (center specific characteristics are described in **Table 2**). The 5-, 10- and 15-year transplant-free survival rates were 90.0%, 79.6% and 66.3% respectively and not significantly different from those observed in the derivation cohort (**Figure 1**).

A comparable overall discriminative ability was found as in the derivation cohort (C statistic 0.82, 95% CI 0.79-0.84). To explore to what extent the GLOBE score might be influenced by the imputation process for missing variables, the discriminative ability of the GLOBE score was additionally tested in cases with complete data. These analysis showed comparable results (C statistic derivation cohort: 0.82, 95% CI 0.78-0.86 and validation: 0.83, 95% CI 0.79-0.86).

The discriminative ability of the GLOBE score was visualised by plotting the transplant-free survival curves for 5 risk groups according to the 10^{th}, 40^{th}, 60^{th} and 90^{th} percentiles of the score (derivation cohort: GLOBE scores -1.26, -0.25, 0.30 and 1.69 respectively and validation cohort: GLOBE scores -1.26, -0.26, 0.30 and 1.75 respectively) (**Figure 3**). Good separation was shown for the survival curves of the 5 risk groups.

There was a good agreement between the curves in the derivation and validation cohort as shown in **Figure 3,** with a good model fit (calibration slope, p=0.64). No re-calibration of the GLOBE score was necessary, when calculating the regression coefficient on the prognostic index (p=0.22). Further, the predicted survival probabilities corresponded well with the observed survival probabilities (**Table 5**).

A



Derivation cohort					
1. <10th perc	249	162	73	21	*Numbers at risk*
2. 10-40th perc	746	509	253	97	
3. 40-60th perc	498	342	187	71	
4. 60-90th perc	747	449	216	82	
5. >90th perc	248	67	10	3	
Validation cohort					
1. <10th perc	154	99	41	17	*Numbers at risk*
2. 10-40th perc	492	339	170	56	
3. 40-60th perc	326	216	114	47	
4. 60-90th perc	474	283	140	43	
5. >90th perc	185	49	10	0	

B

	Derivation cohort			Validation cohort		
	HR	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
<10th percentile	1			1		
10th-40th percentiles	2.26	1.13-4.53	.0214	4.20	1.00-17.59	.0499
40th-60th percentiles	3.18	1.58-6.38	.0011	9.22	2.23-38.16	.0022
60th-90th percentiles	8.96	4.60-17.46	<.0001	25.48	6.30-103.15	<.0001
>90th percentile	58.50	29.87-114.57	<.0001	129.89	31.95-528.05	<.0001

Figure 3. Liver transplant-free survival probability of risk groups according to the GLOBE score
A) Transplant-free survival probability of 5 predefined risk groups according to percentiles of the GLOBE score: (1) <10th, (2) 10th-40th, (3) 40th-60th, (4) 60th-90th and (5) >90th, and B) accompanying hazard ratios between the risk groups in the derivation (N = 2488, solid line) and validation cohort (N = 1631, dotted line).

Table 5. Predicted against observed probability of transplant-free survival in the validation cohort (n=1631)

Risk groups according to percentiles of the GLOBE score	Years of follow-up	Predicted probability[1]	Observed probability[2]
<10th percentile	3-year	0.993	0.993
	5-year	0.988	0.993
	10-year	0.968	0.975
	15-year	0.943	0.975
10th – 40th percentiles	3-year	0.982	0.993
	5-year	0.968	0.985
	10-year	0.918	0.949
	15-year	0.857	0.882
40th – 60th percentiles	3-year	0.965	0.975
	5-year	0.937	0.956
	10-year	0.840	0.864
	15-year	0.732	0.789
60th – 90th percentiles	3-year	0.915	0.924
	5-year	0.854	0.854
	10-year	0.660	0.720
	15-year	0.484	0.478
>90th percentiles	3-year	0.617	0.638
	5-year	0.460	0.474
	10-year	0.183	0.181
	15-year	0.067	0.069

[1]The predicted transplant-free survival probabilities for each risk group were assessed by first applying the GLOBE score of each individual in the validation cohort to the baseline survival estimate $S_0(t)$ derived from the derivation cohort: $S_{GLOBE\ SCORE}(t) = S_0(t)^{\exp(GLOBE\ SCORE)}$. Than, the average of $S_{GLOBE\ score}(t)$ across each risk group was calculated.
[2]The observed probabilities are observed from Kaplan-Meier estimation.

Application of the GLOBE score

An overall threshold was determined for the GLOBE score in the derivation cohort beyond which prognosis of patients significantly deviated from a normal life-expectancy (non-responders). Patients with a GLOBE score above 0.30, which applied to 40% of cases, had a significantly diminished survival compared with a matched general population (hazard ratio (HR) 5.51, 95%CI 4.52-6.72, p<.0001), with 5-, 10- and 15-year transplant-free survival rates of 79.7%, 57.4%, 42.5% respectively. Patients with a GLOBE score of 0.30 or less (responders) had a life-expectancy comparable with a matched general population; the 5-, 10- and 15-year transplant-free survival rates were 98.0%, 92.0%, 82.3% respectively (p<.0001) (**Figure 4**).

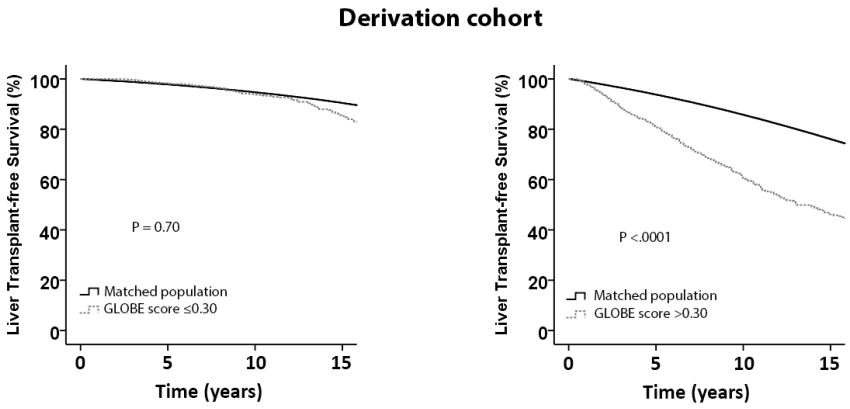


Figure 4. Liver transplant-free survival probability using a GLOBE score threshold
Transplant-free survival probability of patients with a GLOBE score of 0.30 or less compared with an age-, sex- and calendar-time matched population for patients within A) the derivation and C) the validation cohort, and for those with a GLOBE score greater than 0.30 this probability significantly deviated for patients within B) the derivation and D) the validation cohort.

The performance of the GLOBE score was assessed using the aforementioned threshold. A high positive predictive value was found at 5-year follow-up (1057/1084, 98%) and at 10-year follow-up (588/669, 88%), implying that the probability of reaching an adverse outcome is very low for patients identified as a responder. Also a high specificity was found at 5-year follow-up (193/220, 88%) and 10-year follow-up (328/409, 80%) which means that the majority of patients with an adverse outcome were identified as non-responder. Additionally, we found a sensitivity of 65% (1057/1623) at 5-year and 69% at 10-year (588/857) follow-up and a low negative predictive value at 5-year (193/759, 25%) and at 10-year (328/597, 55%) follow-up.

The performance of the GLOBE score compared with other criteria

The overall discriminative ability of the GLOBE score was superior in comparison with previously proposed stratification tools[9-13] **(Table 6)**. To quantify the improvement in discriminative ability the NRI for both events and non-events in the validation set was calculated.[23] The percentage of patients with an event at 5- and 10-year follow-up that were correctly reclassified with the GLOBE score as compared with existing criteria ranged from 3% to 25% and 1% to 22% respectively, and in patients without an event at 5- and 10-year follow-up the NRI ranged from -15% to 18% and -14% to 21% respectively **(Table 7**).

The performance of the GLOBE score among different age groups, disease severity groups and at different time points

Additionally, we created five equal age groups (<45, 45-52, 52-58, 58-66 and ≥66 years), to perform an in-depth analysis of the threshold per age group. Patients within these groups were separately matched with an age- and sex-matched population and thresholds of -0.52, 0.01, 0.60, 1.01 and 1.69 respectively were determined. When using these thresholds 70%, 50%, 30%, 20% and 10% respectively of patients had a diminished survival compared with a matched population. Importantly, this implies that older patients inevitably may derive les impact ultimately from additional therapies.

Within the derivation cohort the performance of the GLOBE score was tested within a subgroup of patients with histological early stage PBC (n=673), defined as stage I or II and a subgroup of patients with histological late stage PBC (n=309), defined as stage III or IV. In the early stage subgroup 280/1090 (26%) patients had a survival significantly deviating from that of a matched population and this were 373/540 (69%) patients in the advanced stage subgroup. In both subgroups the predictive ability of the score was satisfactory with a C statistic of 0.81 (95% CI 0.76-0.86) in the early stage subgroup and 0.78 (95% CI 0.74-0.83) in the late stage. Comparable results were found when repeating these analyses in the validation cohort; with a C statistic in the early stage (n=448) of 0.85 (0.79-0.91) and in the late stage (n=212) of 0.79 (0.72-0.86).

Importantly, the risk score was calculated based on lab values collected 1 y after UDCA therapy, but transplant-free survival could still be accurately calculated by the GLOBE score with laboratory values collected at 2–5 y after treatment (**Table 8**).

Table 6. Performance of biochemical response criteria and the GLOBE score

	Derivation cohort (n=2488)					Validation cohort (n=1631)				
Criteria[a]*	**HR**	**95% CI**	**P-value**	**C statistic**	**95% CI**	**HR**	**95% CI**	**P-value**	**C statistic**	**95% CI**
Barcelona[9]	1.69	1.39-2.06	<.0001	0.58	0.55-0.61	1.84	1.42-2.38	<.0001	0.57	0.54-0.61
Paris-1[10]	3.64	3.03-4.36	<.0001	0.69	0.66-0.71	4.61	3.61-5.90	<.0001	0.70	0.67-0.73
Rotterdam[11]	4.11	3.32-5.08	<.0001	0.69	0.66-0.71	4.10	3.11-5.42	<.0001	0.68	0.65-0.71
Toronto[12, b]	2.13	1.76-2.56	<.0001	0.61	0.58-0.63	2.46	1.90-3.18	<.0001	0.62	0.59-0.65
Paris-2[13]	2.82	2.29-3.47	<.0001	0.63	0.61-0.65	2.89	2.17-3.85	<.0001	0.63	0.61-0.66
GLOBE score	-	-	-	0.81	0.79-0.83	-	-	-	0.82	0.79-0.84

[a]Response assessed after one year UDCA treatment. Response according to Toronto criteria calculated after 2 years.
[b]After 2 years follow-up 2335/2488 patients of the derivation cohort and 1521/1631 patients of the validation cohort were at risk.

Table 7. Net reclassification improvement of the GLOBE score compared with existing response criteria for events and non-events at 5-year follow-up

	Derivation cohort				Validation cohort			
	5-year		**10-year**		**5-year**		**10-year**	
Criteria[a]	*Events NRI[b]*	*Non-events NRI[b]*	*Events NRI[b]*	*Non-events NRI[b]*	*Events NRI[b]*	*Non-events NRI[b]*	*Events NRI[b]*	*Non-events NRI[b]*
Barcelona	25%	10%	21%	13%	26%	9%	22%	12%
Paris-1	12%	-8%	15%	-6%	17%	-7%	13%	-4%
Rotterdam	21%	-15%	22%	-14%	23%	-13%	23%	-13%
Toronto	21%	2%	21%	6%	28%	0%	20%	4%
Paris-2	3%	18%	1%	21%	5%	18%	0%	21%

Abbreviation: NRI, net reclassification improvement
[a]All criteria were calculated after 1 year follow-up except Toronto criteria which was calculated after 2 years follow-up.
[b]The event NRI and non-event NRI were calculated as following: event NRI = (number of events classified up – number of events classified down) / number of events and non-event NRI = (number of non-events classified down – number of non-events classified up) / number of non-events.[22]

Table 8. Predictive performance of the GLOBE score calculated after *n* years of UDCA therapy

		Validation cohort n=1630
Follow-up	**C statistic**	**95% CI**
1 year	0.82	0.79-0.84
2 years	0.83	0.80-0.85
3 years	0.83	0.80-0.85
4 years	0.83	0.80-0.86
5 years	0.84	0.81-0.87

DISCUSSION

In this study of over 4000 UDCA-treated patients with PBC from across Europe and North America we present the GLOBE score, an internationally relevant and validated risk assessment tool, able to accurately stratify patients to high and low risk. The score comprises five simple, readily available and objective variables: age, bilirubin, albumin, alkaline phosphatase and platelet count. Moreover, through robust evaluation and validation we demonstrate appropriate test characteristics in subgroups with early and advanced disease. Most importantly, the prognostic ability of the score was found to be markedly superior to previously proposed criteria for (non-)response to UDCA. The score has utility for patients managed with PBC internationally, as a means to more readily stratify risk of adverse outcomes, and hence tailor patient education. In particular, in an era of potential new therapies the GLOBE score is better able than current stratification tools to highlight patients at greatest need for new therapies. Of further relevance to the health economics of PBC, the GLOBE score improves capacity to identify individuals in whom UDCA monotherapy should be continued, with opportunities to de-escalate care back to their primary care provider.

Previous studies have extensively documented the prognostic importance of the individual components of the GLOBE score. In particular, age, bilirubin and albumin have been recognized as important predictors of survival in PBC, irrespective of UDCA treatment[7, 8, 25, 26] In general, age and mortality are strongly correlated and not surprisingly age proved to be an independent predictor of liver transplantation or death in present study. Serum bilirubin is generally considered the strongest and most independent predictor of outcome in PBC,[18, 27-29] and is a main component of prognostic models[25, 30-32] and response criteria in PBC.[10, 11, 13, 33] Serum bilirubin levels normally increase relatively late in the course of disease. However, its predictive value is not limited to late stage disease, as suggested by our previous finding that even in patients with normal levels, prognosis improves as levels fall.[18] Alkaline phosphatase levels are of key importance in establishing the diagnosis PBC.[2, 3] Changes in alkaline phosphatase levels have previously been documented to provide

significant prognostic information, both in UDCA-treated[9, 10, 12, 13, 18, 34] and non-treated PBC.[18] Finally, the platelet count, generally considered as a marker of portal hypertension,[35] has been validated as an independent predictor of outcome in addition to current biochemical response criteria.[15, 36]

Although some of the factors comprising the score, such as bilirubin and albumin, will change relatively late in the course of disease, the GLOBE score performed well in patients with early stage disease. This is probably largely explained by the well-documented strong predictive significance of alkaline phosphatase values, even in cases with normal bilirubin.[18]

Our score provides improved identification of patients insufficiently responding to UDCA in comparison with previously reported criteria (**Table 6**). As reflected by the high positive predictive value, responders to UDCA according to the GLOBE score are at low risk for future adverse events. Therefore these patients can reliably be advised to continue with UDCA mono-therapy. The GLOBE score also allows more reliable identification of patients likely to have a future unfavourable health outcome. Thus, for healthcare providers the GLOBE score provides an improved instrument for selecting candidate patients for additional, second-line therapies. The superior performance of our score is likely attributable to the effect of dichotomization of every single variable in previously proposed response criteria. Dichotomization of continuous variables inevitably will have led to loss of predictive ability.[37] Moreover, age, as a recognized major predictor of survival, was included in our score. Importantly, we confirm that younger patients have the potential to benefit more from additional PBC therapies than older patients.[14] Finally, the methodological approach to base the score on a prognostic index, corresponding with a continuum of possible outcomes, is an important factor explaining improved ability to reliably estimate prognosis using the GLOBE score.

Other predictors of outcome in PBC have been suggested, including liver histology and elastography.[38, 39] Liver histology has important prognostic meaning,[38] but in the majority of cases liver biopsy is not considered necessary for diagnosis.[3] Moreover, given other disadvantages, such as its invasive character, sampling error and inter-observer variation, liver biopsy is no longer routinely performed in the management of PBC patients. Non-invasive assessment of liver fibrosis with transient elastography is an interesting alternative,[39] but data supporting this technique as an important clinical tool are still limited and further validation is required. Elastography may be less suitable for assessing the response to medical treatment, especially after a relatively short duration of treatment, as PBC is a slowly progressive disease, suggesting it might take longer before reliably detectable changes in liver stiffness will ensue.[4-6] Biochemical markers are routinely checked during yearly check-up of PBC patients, and levels of biochemical variables after a short period of UDCA treatment are strongly associated with long-term outcome.[9-13, 18, 34] Considering the fact that biochemical markers are easily obtainable and readily available, they seem more attractive and preferable for first-line patient stratification.

A potential limitation to our study is the use of reference population data originating from only one country, namely the Netherlands, for developing the Global PBC Study Group Score. However, according to life table data of the World Health Organisation (WHO) life expectancy was comparable among the countries involved in this study.[40] Therefore, this may not be a factor of major relevance. Further, we were not able to take into account other laboratory variables of potential interest in PBC, such as gamma-GT, IgM, IgG and prothrombin time. Due to the nature of our study laboratory data were also not always fully complete, especially when inclusion in the original cohort studies occurred more than 15-20 years ago. However, considering the exceptionally large dataset, we believe our results are sufficiently robust, as well as notably representative. Finally, the reliability of our findings is supported by the validation of the prognostic model in a separate population of considerable size. The complex calculation of the GLOBE score has been simplified by the development of a web application to improve its usage in clinical practice (www.globalpbc.com).

In conclusion, we demonstrate that the prognosis of patients with PBC, irrespective of the stage of disease, who have been treated with UDCA for one year can be readily determined using a *de novo* derived and validated, risk calculation. Our score performs significantly better than thus far proposed criteria for response to UDCA thereby providing internationally representative data to quantify the needs of low- and high-risk patients with PBC. The GLOBE score therefore complements efforts to develop and implement a more stratified, evidence-based, approach to the care of patients with PBC.

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CHAPTER 5

Stratification of hepatocellular carcinoma risk in primary biliary cirrhosis: a multicenter international study

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Gut 2015; epub ahead of print

ABSTRACT

Background & Aim: Hepatocellular carcinoma (HCC) is an infrequent yet critical event in primary biliary cirrhosis (PBC); however, predictive tools remain ill-defined. Our objective was to identify candidate risk factors for HCC development in patients with PBC.

Methods: Risk factor analysis was performed in over 15 centres from North America and Europe spanning >40 years observation period using Cox proportional hazards assumptions, logistic regression, and Kaplan-Meier estimates.

Results: Of 4565 patients with PBC 123 developed HCC, yielding an incidence rate (IR) of 3.4 cases/1000 patient-years. HCC was significantly more common in men (p<.0001), and on univariate analysis factors at PBC diagnosis associated with future HCC development were male sex (unadjusted HR 2.91, p<.0001), elevated serum aspartate transaminase (HR 1.24, p<.0001), advanced disease (HR 2.72, p=.022), thrombocytopenia (HR 1.65, p<.0001), and hepatic decompensation (HR 9.89, p<.0001). As such, non-treatment with ursodeoxycholic acid itself was not associated with cancer development; however, 12-month stratification by biochemical non-response (Paris-I criteria) associated significantly with future risk of HCC (HR 4.52, p<.0001; IR 6.6 vs 1.4, p<.0001). Non-response predicted future risk in patients with early stage disease (IR 4.7 vs 1.2, p=.005), advanced disease (HR 2.79, p=.02; IR 11.2 vs 4.4, p=.033), and when restricting the analysis to only male patients (HR 4.44, p<.001; IR 18.2 vs 5.4, p<.001). On multivariable analysis biochemical non-response remained the most significant factor predictive of future HCC risk (adjusted HR 3.44, p<.0001).

Conclusions: This uniquely powered, internationally representative cohort robustly demonstrates that 12-month biochemical non-response is associated with increased future risk of developing HCC in PBC. Such risk stratification is relevant to patient care and development of new therapies.

INTRODUCTION

Primary biliary cirrhosis (PBC) is the most prevalent autoimmune liver disease, characterised by ductopenia, cholestasis and a risk of progressive liver fibrosis. Life expectancy is reduced in PBC and prognosis largely dictated by development of cirrhosis and portal hypertension,[1-3] including for some the development of hepatocellular carcinoma (HCC). Given the relative infrequency of PBC compared to other chronic liver diseases, large scale, robust and representative analyses of HCC risk in PBC remain limited.

Presently, the majority of patients with PBC are treated with ursodeoxycholic acid (UDCA).[1,4] Treatment benefit is best highlighted by applying biochemical stratification to therapy,[3,5-9] and up to two-thirds of patients have an improved transplant-free/overall survival in this regard. However, there is a paucity of data with regard to biochemical response and modification of cancer risk in PBC.[10,11] Consequently it remains unclear how much risk/benefit there is in performing HCC surveillance for patients with well-treated disease.[12] Across the spectrum of liver disease generally, HCC incidence appears greatest among individuals with advanced fibrosis/cirrhosis, particularly men;[13] however, such observations in PBC frequently represent single centre studies, or are not immediately generalisable to Western practice.[7,10,14-24] For example, a recent nationwide population survey from Japan identified a sex-specific contribution to HCC risk with respect to disease stage in Japanese patients with PBC.[24]

To address and overcome limitations to current knowledge and practice, we now describe the incidence of HCC across a global PBC cohort, with the specific aim of identifying predictive factors in a robust, statistically powered and internationally representative population. In so doing we document critical insight into challenges pertaining to long term patient follow-up, hepatocellular cancer susceptibility and disease outcome. Furthermore we identify a protective association in patients meeting specific biochemical endpoints that can be used to stratify HCC risk in the clinic setting.

PATIENTS AND METHODS

Study setting and design

We collected and analysed data from well-characterised patients with an established diagnosis of PBC,[1,4] who had previously attended or were under current clinical follow-up between 1959 and 2012. Our catchment population comprised over 15 centres across the UK, Europe, the USA and Canada, as detailed elsewhere.[25] Both UDCA-treated and non-treated individuals with an established diagnosis of PBC in accordance with European and American guidelines were eligible for inclusion. Individual centre datasets (ICDs) contained

mostly prospectively collected follow-up data on patients from diagnosis and/or start of UDCA therapy. Upon study initiation, ICDs were transferred onto a standardised case record form formulated by the Global PBC Study Group committee and amalgamated onto a common 'master' database for downstream analysis. Individual clinical and laboratory characteristics pertained to gender, clinician reported age and date of PBC diagnosis, liver histology, UDCA treatment (start date, dosage and duration of therapy), antimitochondrial antibody serology, laboratory values (serum alanine transaminase, aspartate transaminase (AST), alkaline phosphatase, bilirubin, albumin and platelets) at PBC diagnosis and annually thereafter, date of HCC diagnosis, and liver transplantation and mortality status.

Baseline was set as the point of starting therapy for patients in receipt of UDCA ≥12 months; and the date of first centre visit in non-treated individuals. Adopted biochemical response criteria for this study were as previously documented; specifically those from Barcelona,[6] Paris,[5,8] Rotterdam[7] and Toronto.[9] Liver biopsy performed within 1 year of study entry was classified as baseline histological assessment. Tissue material was assessed for liver disease severity according to the Ludwig and Scheuer classification, and staged histologically as early (I/II) or late (III/IV). Any individual with evidence of cirrhosis before confirmed PBC diagnosis was classified as cirrhotic at baseline. Given that liver biopsy is no longer standard practice in PBC, advanced baseline disease was also deemed present according to clinical features (eg, hepatic decompensation) and/or biochemical indices as per the criteria established by ter Borg et al.[26]

Individual patient follow-up was as per centre-specific practice, which although slightly variable included a clinic review at a minimum of every 12 months in the absence of cirrhosis, and at 6-monthly intervals for patients with evidence of advanced disease. Surveillance for HCC was conducted according to accepted international protocols, specifically 6-monthly ultrasound ± serum α-fetoprotein quantification. Confirmatory diagnosis was as per (timeline specific) internationally accepted protocols, either: (a) histopathological confirmation; (b) two coincident imaging techniques (CT, MRI, or contrast-enhanced ultrasonography) showing a focal lesion >2 cm with arterial phase enhancement; or (c) one imaging technique showing a focal lesion >2 cm with arterial phase enhancement in the presence of an α-fetoprotein serum titre >400 ng/mL.

Quality control

Individuals were excluded from analysis if follow-up data were insufficient or unavailable (<6 months' follow-up or <2 clinic visits recorded), the start date of treatment or the exact date of major clinical events was unknown, and in the event of confirmed past/concomitant hepatitis B (HBV) or hepatitis C virus (HCV) infection, Wilson disease, α-1 antitrypsin deficiency, hereditary haemochromatosis, alcoholic liver disease, or overt overlapping features with autoimmune hepatitis.

Completeness, plausibility and validity of the data were carefully verified (by PJT, WJL and BEH). Extensive efforts, including individual site-centre visits (WJL) with personalised objective review of historical medical charts, were undertaken to retrieve missing data. Data pertaining to all cohorts were collected through to the end of December 2012.

Statistical analysis

The primary endpoint of this study was defined as development of HCC, and patients without a clinical event in this regard were censored at date of last follow-up, liver transplantation or death. Univariate and multivariable Cox proportional hazards models were fit in order to assess the impact of individual covariates on the instantaneous rate of events, with time-to-event analysis also being ascertained through Kaplan-Meier estimates. In order to account for possible heterogeneity across centre-specific populations, analyses were further stratified by centre.

Data are presented using the median and interquartile range for continuous variables and a value of $P<.05$ considered statistically significant. Analyses were conducted using SPSS Statistics V.22.0 (SPSS Inc, Chicago, Illinois, USA), and repeated in a multiple imputed database to validate findings and study any potential bias. SAS V.9.0 (SAS Institute, Cary, North Carolina, USA) was used to generate 10 imputed datasets before combining results and retesting of multivariable analysis.

RESULTS

Characteristics of the patient population

Our study cohort comprised 4845 patients (**Figure 1A** and see **Supplementary Table 1**); however, those without clear documentation of HCC status (presence or absence thereof) during follow-up were exempt from further analysis, as were individuals diagnosed with HCC simultaneously or within 12 months of PBC diagnosis. Therefore the final working group consisted of 4565 patients with PBC (90% female), of which 123 developed evidence of HCC during their clinical course, yielding an actuarial incidence rate of 3.4 cases/1000-patient years (**Figure 1B**).

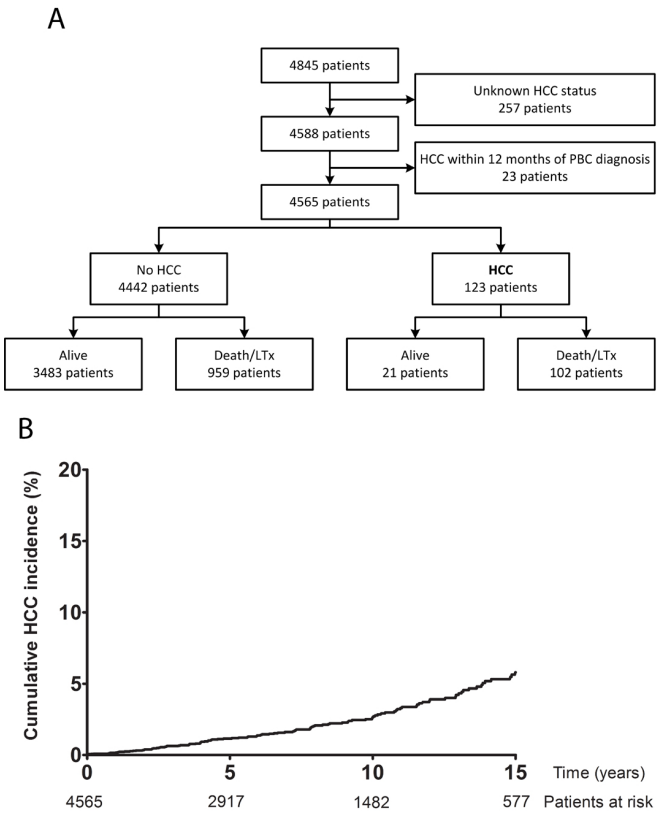


Figure 1. Study cohort
At time of analysis (A) data were available from 4845 patients; however, 257 patients were excluded given that documentation of HCC status (presence or absence thereof) was not recorded in medical records. A further 23 patients were excluded given that they were diagnosed at the same time (within the 1st year) of PBC diagnosis and identification of risk factors pertaining to HCC risk would have been inaccurate. The final study group therefore consisted of 4565 patients (B) of which 123 developed HCC over 36,577 patient years.

In PBC, HCC incidence is increased in men and patients with advanced disease at baseline

Development of HCC was associated with significantly poorer transplant-free and overall survival (hazard ratio [HR] 22.61, 95% CI 18.34 to 27.87; P<.0001), and at baseline future risk of HCC was increased particularly in patients of male gender (**Figure 2A** and **Supplementary Table 2**) and those having moderate/late (advanced) biochemical disease (**Figure 2B**). Men were observed to have a significantly greater incidence of HCC when analysis was restricted to patients with advanced disease (**Supplementary Figure 1**), and gender remained a significant risk factor following multivariable adjustment of baseline covariates (**Supplementary Table 2**). Subsequent transplant-free survival following development of cancer was not, however, different between men and women (**Supplementary Figure 2**).

Overall, the proportion of patients with histological evidence of advanced fibrosis at PBC diagnosis was also significantly greater in patients who developed HCC (P<.0001); but the absence of baseline liver biopsy in many of our study group (n=2685) precluded further, more explicit analysis pertaining to histological characteristics.

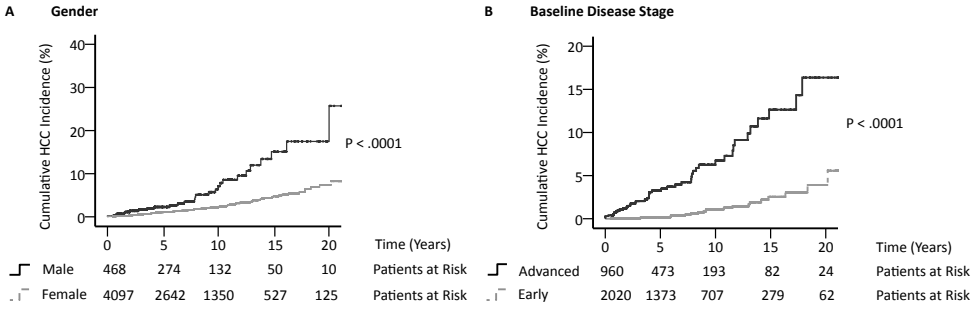


Figure 2. Cumulative HCC incidence according to gender and baseline PBC disease severity
Kaplan Meier estimates of HCC incidence in: (A) Men vs. women, 6.7 vs. 2.6 cases per-1,000 patient years; and (B) patients with advanced vs. early disease, 7.6 vs. 1.3 cases per-1,000 patient years (according to criteria by ter Borg et al. [26]).

Biochemical non-response predicts future risk of HCC in PBC patients

Overall, 96% (n=4361) of individuals remained under follow-up for a minimum of 12 months. Analysing this cohort in its entirety, 85% (n=3724) of patients were in receipt of UDCA therapy for ≥1 year, in keeping with the demographic of PBC treatment reported elsewhere.[27] No significant difference between HCC appearance rates was observed, however, between treated versus non-treated patients (P=.972; **Supplementary Figure 3**).

Twelve-month biochemical response (Paris-I) was calculable in 65% (n=2425) of all treated individuals and met by an inclusive 72% (n=1734). On univariate analysis HCC risk was observed to be significantly greater in biochemical non-responders according to Paris-I, Paris-II, Rotterdam and Toronto (**Table 1 and Figure 3**) but not Barcelona criteria (HR 1.48, 95% CI 0.93 to 2.36; P=.099). Furthermore, biochemical non-response retained its predictive value when analysis was inclusive of patients not receiving UDCA (**Supplementary Figure 4**), and when extending the period over which response was assessed from 1 to 2 years (calculable in n=2725 (73%) of all UDCA-treated patients—HR 4.41, 95% CI 2.60 to 7.47; P<.0001; **Supplementary Figure 5**). Given that the most widely adopted criterion in clinical practice is Paris-I,[3,27,28] this model is used to classify biochemical response in subsequent discussion unless otherwise specified.

Table 1. Covariates associated with future HCC-risk following 12 months of UDCA treatment

	Univariate analysis		Multivariable analysis*		Multivariable analysis following multiple imputation*	
	Hazard ratio (95% CI)	**P-value**	**Adjusted hazard ratio (95% CI)**	**P-value**	**Adjusted hazard ratio (95% CI)**	**P-value**
a) UDCA treated patients only						
Advancing age (per 10-yrs.)**	1.21 (1.34-1.41)	.022	-	n.s.	1.31 (1.08-1.57)	.009
Male gender	2.91 (1.90-4.48)	<.0001	-	n.s.	2.41 (1.50-3.86)	<.0001
Advanced disease**	2.72 (1.43-5.18)	.022	-	n.s.	-	n.s.
Thrombocytopenia at 12 mths. (per $50x10^3/mm^3$ decline)	1.65 (1.35-2.02)	<.0001	1.42 (1.10-1.74)	.003	1.42 (1.25-1.61)	<.0001
Paris I not fulfilled	4.52 (2.59-7.94)	<.0001	3.44 (1.65-7.14)	<.0001	3.42 (2.22-5.26)	<.0001
Paris II not fulfilled	3.57 (1.83-6.94)	<.0001	-		-	
Rotterdam not fulfilled	2.22 (3.82-12.50)	<.0001	-		-	
Toronto not fulfilled***	1.89 (1.13-3.16)	.016	-		-	
b) UDCA treated and non-treated patients**						
Thrombocytopenia at 12 mths. (per $50x10^3/mm^3$ decline)	1.65 (1.35-2.02)	<.0001	1.35 (1.05-1.65)	.003	1.41 (1.25-1.58)	<.0001
Paris I not fulfilled****	4.76 (2.82-8.00)	<.0001	3.82 (1.86-7.81)	<.0001	3.23 (2.14-4.86)	<.0001
Paris II not fulfilled****	3.48 (1.88-6.45)	<.0001	-		-	
Rotterdam not fulfilled****	6.62 (3.80-11.49)	<.0001	-		-	
Toronto not fulfilled****	1.92 (1.18-3.13)	.009	-		-	

* Also adjusted for center-specific heterogeneity.
** At time of PBC diagnosis.
*** Calculated at 2 years. [9]
**** Inclusive of patients not in receipt of UDCA for whom biochemical response was calculable 1-year following study entry

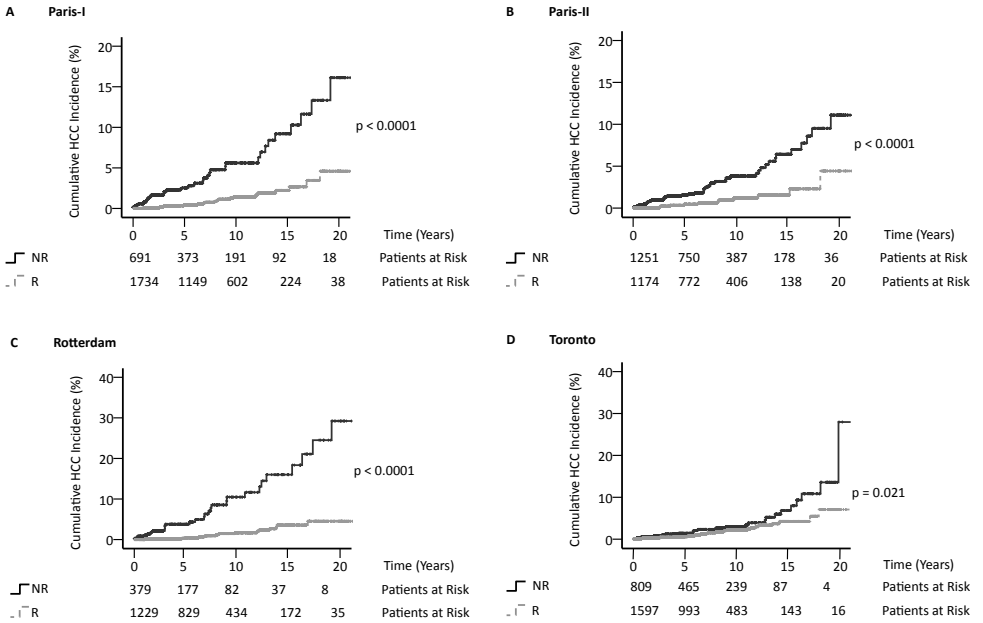


Figure 3. Cumulative HCC appearance rates according to biochemical response
Kaplan Meier estimates of HCC incidence in biochemical non-responders (NR) versus responders (R) as per the following criteria: (A) Paris I, 6.6 vs. 1.4 cases per-1,000 patient years; (B) Paris II, 4.3 vs. 1.2 cases per 1,000 patient years; (C) Rotterdam, 11.9 vs. 1.8 cases per 1,000 patient years; and (D) Toronto, 4.5 vs. 2.4 cases per 1,000 patient years. No significant differences were observed when biochemical response was stratified using Barcelona criteria (data not presented).
Analysis restricted to UDCA-treated patients only in whom 12-month biochemical data available to calculate response (24-months in the case of Toronto criteria [9]). Time measured in years following calculation of biochemical response.

Biochemical non-response predicts added HCC risk in men with PBC

Having identified that HCC developed more frequently in male patients compared to females, we next analysed the performance of biochemical response criteria specifically in men with PBC. Indeed, HCC incidence was greater in male non-responders versus responders (HR 4.44, 95% CI 1.29 to 10.20; P<.001) (**Figure 4**), and the former represented the group at highest future risk.

Given that gender and advanced disease may adversely influence attainment of satisfactory biochemical response,[27,29] an assessment of interaction terms was conducted. Although a greater proportion of men relative to women were observed to have advanced baseline disease (**Supplementary Figure 1**), no significant interactions between gender and disease stage (P=.346), gender and biochemical response (P=.285) or biochemical response and disease stage (P=.690) were identified; thus strengthening the independent value of biochemical non-response in predicting future HCC risk.

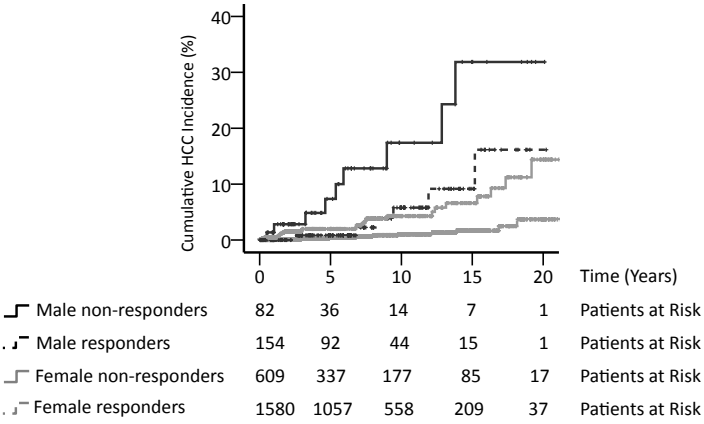


Figure 4. HCC incidence stratified according to gender and biochemical response
Kaplan Meier plot comparing cumulative HCC incidence in male non-responders vs. female non-responders (HR: 3.70, 1.71-8.00, P<.001; 18.2 vs. 5.2 cases per 1,000 patient years, log-rank P<.001); male non-responders vs. male responders (HR: 4.44, P<.001; 18.2 vs. 5.4 cases per 1,000 patient years, log-rank P<.001); male responders vs. female non-responders (HR: 0.90, 0.36-2.21, P=.811; 5.4 vs. 5.2 cases per 1,000 patient years. log-rank P=.766); female non-responders vs. female responders (HR: 4.74, 2.44-9.22, P<.0001; 5.2 vs. 1.1 cases per-1,000 patient years, log-rank P<.0001); and male responders vs. female responders (HR: 5.29, 2.03-13.78, P<.001; 5.4 cases vs.1.1 cases per-1,000 patient years, log-rank P=.0001).
Analysis conducted in UDCA-treated patients only, for whom 12-month biochemical data was available to calculate response. Time measured in years following calculation of biochemical response.

Biochemical non-response predicts HCC risk irrespective of baseline PBC disease stage

As HCC most often develops on a background of severe fibrosis/cirrhosis—a factor associated with reduced biochemical response to treatment[29]—subsequent analysis focused exclusively on patients with evidence of advanced hepatic disease at time of PBC diagnosis. Within this inherently high risk population, stratification through biochemical response identified that the subgroup at greatest hazard of HCC fell into the non-response category (HR 2.79, 95% CI 1.18 to 6.94; P=.02), whereas in biochemical responders of matched disease stage, development of HCC was much less apparent (**Figure 5A**). Although fewer patients with advanced disease at baseline were exposed to UDCA therapy (**Supplementary Table 3**), when the cohort was extended to include non-UDCA-treated patients, biochemical non-response was still able to identify those at increased future HCC risk (**Supplementary Figure 6A**).

Given reports of HCC developing in the absence of advanced fibrosis,[15,24] thereafter we conducted an evaluation of risk factors exclusively in patients presenting with early-stage PBC at baseline. Across this subgroup, HCC developed infrequently and as a relatively late event (**Figure 5B and Supplementary Figure 6B**); however, future HCC risk was observed to be significantly greater in patients who failed to achieve 12-month biochemical response (P=.005).

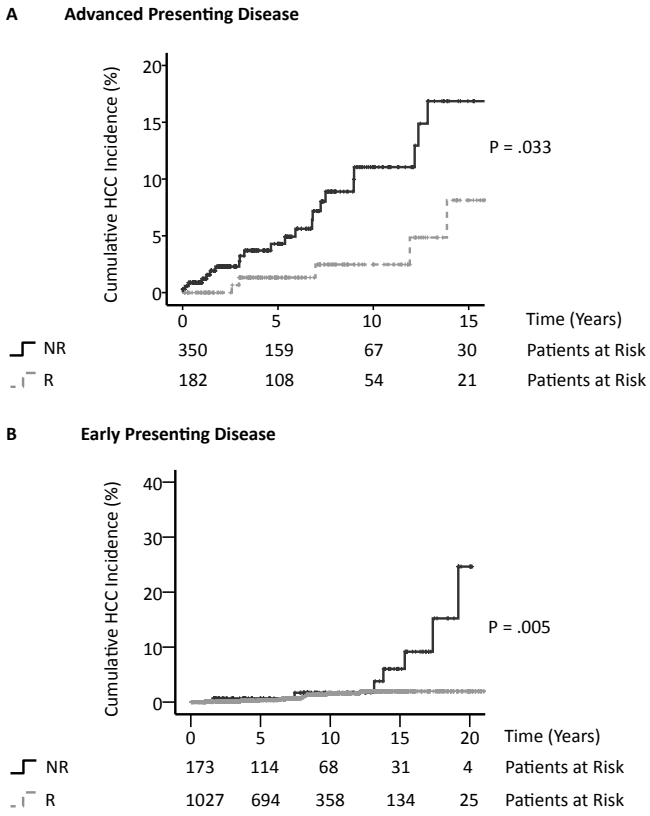


Figure 5. HCC incidence in patients with varying disease stage stratified according to biochemical response

Kaplan Meier estimate restricted to those with: (A) Advanced presenting disease and biochemical non-response (NR) versus response (R), 11.2 vs. 4.4 cases per 1,000 patient years; and (B) Early presenting disease and biochemical non-response versus response, 4.7 vs. 1.2 cases per 1,000 patient years. Analysis conducted in UDCA-treated patients only in whom 12-month biochemical data available to calculate response. Time measured in years following calculation of biochemical response.

Biochemical non-response remains the most significant predictor of future HCC risk in PBC

When performing a multivariable analysis of all hitherto identified risk factors—both those present at time of PBC diagnosis as well as over time—only thrombocytopenia and biochemical response retained statistical significance (**Table 1**). This observation held true when extending the analysis to include patients not receiving UDCA as well as when the model was adjusted for centre-specific heterogeneity.

In order to confirm an absence of bias from missing data we next performed multiple imputation analysis (**Supplementary Figure 7**).[30] In doing so, biochemical non-response was validated as an independent and significant predictor of future HCC risk (**Table 1 and Figure 6**).

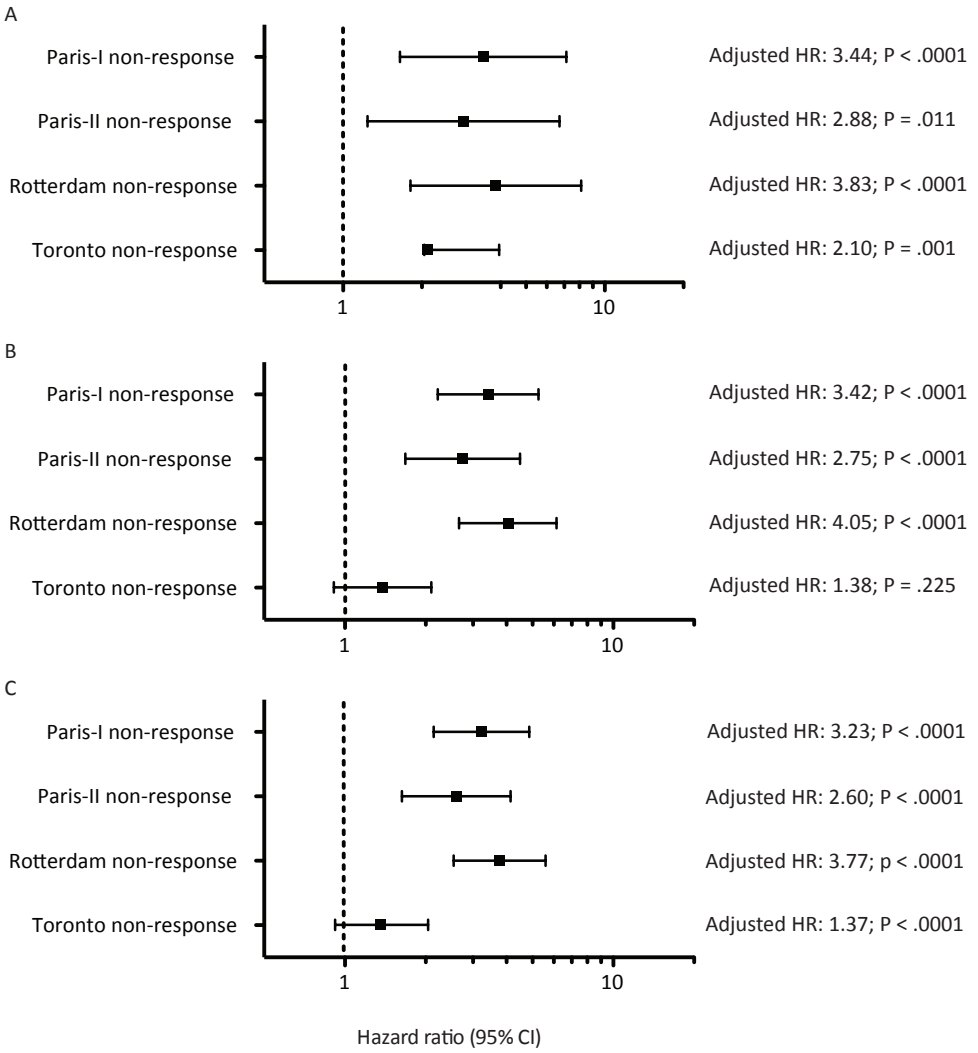


Figure 6. Comparative (multivariable) evaluation of biochemical response in predicting HCC risk
Performance of biochemical response criteria in predicting future HCC risk following: (A) Individual multivariable analysis (stepwise backward model) adjusted for age at PBC diagnosis, sex, disease stage, hepatic decompensation, remaining laboratory parameters (not included in respective biochemical response criteria) and centre-specific heterogeneity; (B) Analysis as in A following multiple imputation to correct for missing data values – UDCA treated patients only; and (C) Analysis as in B following inclusion of non-UDCA untreated patients in whom 12-month biochemical response criteria was calculable.

DISCUSSION

We report a robust and uniquely powered evaluation of the largest internationally representative PBC cohort assembled to date, with the specific aim of identifying risk factors for HCC. In doing so we demonstrate that HCC is a critical event in the clinical course of PBC and associated with significantly poorer transplant-free survival. Although the incidence is significantly greater in male patients and those with advanced disease, 12-month biochemical non-response represents an independent and additive predictor for HCC, and on performing a comparative assessment of all identified covariates remained the most significant risk factor.

Historically, development of HCC in PBC was considered to be predominantly restricted to men, with a more variable correlation reported for disease stage.[10,11,14,17,21,22] Initially perceived as a relatively rare complication, some report that the incidence is in fact comparable to chronic HCV infection,[20,31] and indeed we found a similar frequency of HCC in PBC as previously documented.[16] Larger, more contemporary reports also describe an association with male gender,[24] as well as older age and advanced histological disease. However, the relatively low number of patients included in several earlier studies, as well as restricted geographical influence across others, have yielded many inconsistent results.

In agreement with preceding investigators we now robustly validate male sex as a risk factor,[21,24,32] although on further analysis statistically significant differences between genders appear restricted to patients with advanced disease at presentation. Although suggestive that as a subgroup men are more likely to be diagnosed with PBC at a later stage,[27] advanced disease was not found to be an interaction moderator in this regard. Prior studies have also documented relatively higher rates of past HBV infection and alcohol consumption in men.[24] Despite being considered as exclusion criteria in our study, the complete distribution of past viral infection by gender across our global cohort could not be obtained, and viral serology would not have been routinely tested in patients diagnosed with PBC pre-1980. The reason for increased hepatocarcinogenesis in male patients across a disease with such overwhelming female predisposition remains unknown, although may be due to a lack of protective effect from oestrogen-mediated inhibition on specific cytokines such as interleukin-6.[33] This however remains speculative.

In addition to male gender, advanced biochemical disease and thrombocytopenia were factors identifiable at PBC diagnosis associated with future risk of HCC—in keeping with many early reports wherein tumours developed exclusively on a background of late-stage disease.[14,17,21,22,34] The majority of patients in our cohort received UDCA, and the effect of PBC-specific therapy with regard to HCC risk represented an understudied topic.[10,11] UDCA therapy per se had no apparent effect on risk of HCC development across our cohort; but when stratifying according to several biochemical response criteria, patients

classified as non-responders developed significantly more HCC during their clinical course. Indeed, risk was significantly greater in biochemical non-responders and this observation retained significance when inclusive of patients who never received UDCA but for whom categorisation of response was possible. It is likely therefore that achievement of biochemical response according to specific criteria—irrespective of whether this occurs in the context of therapy—infers a surrogate associated with improved HCC-free survival rather than a chemo-preventative effect of UDCA. The present evaluation not only validated findings of a previous much smaller study,[11] but on performing a sub-analysis in patients identified as inherently high risk (men and those with advanced disease), biochemical non-response was associated with additional future risk of HCC. Indeed, on multivariable analysis only thrombocytopenia and biochemical non-response retained statistical significance and superseded the effect of gender and other tested parameters of disease stage. Of interest, platelet count is commonly employed as a surrogate of portal hypertension,[35] and as part of the AST/platelet ratio has recently been validated as an independent and additive biomarker of transplant-free survival in biochemical responders.[3] However, given the relatively small number of HCC observed in responding patients, substratification of cancer risk in such regard would prove difficult.

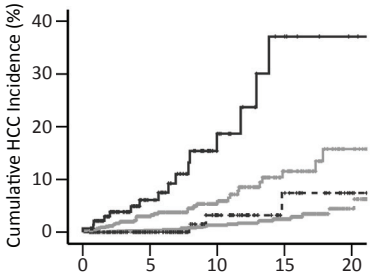
The lower incidence in patients achieving biochemical response has practical implications for HCC surveillance (**Supplementary Table 4**), which at present is advocated for all chronic liver disease patients in which the annual incidence approaches or exceeds 1.5%.[12,36] Specific to PBC, surveillance recommendations apply to all with advanced histological disease; however, in the present day clinical setting liver biopsy is rarely performed, and the advent of progressive imaging techniques makes tissue analysis rarely necessary in the 'diagnostic work-up' of suspected HCC.[13,36] The use of transient elastography as an alternative risk marker of disease stage is also evolving,[37] although due to limited availability still requires aetiology-specific validation. The prevalence of a globally aging population represents a further impending burden on healthcare services. In this regard, older age is increasingly recognised to confer additional HCC risk;[38,39] the average age at time of HCC diagnosis being 67 years across our study population.

The emergence of well-substantiated treatment response criteria has allowed accurate prediction of transplant-free survival in patients with PBC,[3,5-9,27] and herein we illustrate that failure to achieve the same biochemical endpoints confers increased HCC risk. Moreover, the relatively low incidence among those who achieve adequate biochemical response, even in men and individuals with evidence of advanced presenting disease, questions routine HCC surveillance in well-treated patients irrespective of gender and disease stage. Therefore, we recommend particular attention to: (1) male patients who either fail to achieve biochemical response (irrespective of disease stage), or in whom cirrhosis is already established (irrespective of biochemical response status); and (2) all female non-responders with evidence of advanced disease.

As with any longitudinal study evaluating long term outcomes, some patient data were inevitably not available during follow-up, whereas censoring at time of liver transplantation or death (free of HCC) may have led to pre-selection bias by restricting inclusion of patients who survive without transplant. However, such patients are no longer at risk of native liver HCC over time, and this approach is commonly adopted in studies where transplant-free mortality is not the primary endpoint. Moreover, all explanted livers were rigorously examined for the presence of HCC irrespective of transplant indication. In addition, our overall large sample size as well as validation of results through multiple imputation (**Table 1**; **Figure 6** and **Supplementary Figure 7**) demonstrate that any missing data have not introduced meaningful bias. Nevertheless, it is plausible that a proportion of patients who died during follow-up may have had undiagnosed HCC, and complete post-mortem data in this regard are not possible to obtain. Another limitation to our study is that disease stage was assessed non-invasively for the most part—a reflection of the current standard of care in PBC. Further external validation of our results in this regard is important, albeit accepting the considerable challenges to long term prospective studies in PBC: slowly progressive and uncommon, with currently only one established treatment, and an absence of routine histological evaluation as part of standard clinical practice. We were also unable to obtain data pertaining to smoking status and comorbidities/coexisting extrahepatic autoimmune diseases;[40] however, many previous studies have failed to confirm an association with HCC in this regard.[16,24] While the demography of our cohort is consistent with prior reports, we recognise that studies such as ours are heterogeneous with respect to referral practice between centres and countries. Opportunities therefore continue for large scale population studies in PBC to confirm our findings and better define strategies for clinical practice.

In conclusion, based on our results from the largest multicentre international study to date, we report that HCC is a rare yet critical event in the clinical course of PBC, and one associated with significantly poorer outcome. While the frequency of HCC was increased in men and those with advanced disease, biochemical non-response remained the most important risk factor, retaining predictive value independently and additively of disease stage and patient gender. Our globally representative data therefore add new knowledge to HCC risk in PBC and informs ongoing discussions about stratified treatment and surveillance. Additionally, given the potential use of surrogate endpoints in development of new therapies beyond UDCA, our data lend support to a meaningful reduction in HCC risk for PBC patients meeting specific biochemical response criteria.

SUPPLEMENTARY FIGURES

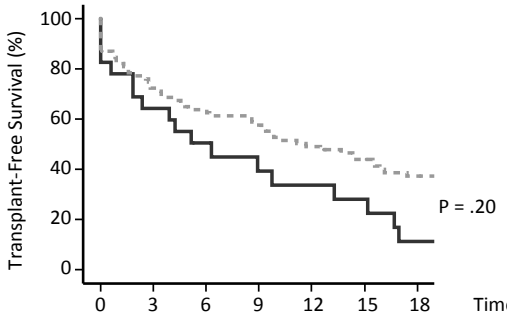


Males; adv. disease	142	70	25	8	2	Patients at Risk
Females; adv. disease	818	407	169	73	21	Patients at Risk
Males; early disease	159	98	49	22	6	Patients at Risk
Females; early disease	1861	1279	659	258	56	Patients at Risk

Supplementary Figure 1. HCC incidence stratified according to gender and disease stage at presentation

Kaplan Meier estimates illustrating differences of HCC incidence rate in male patients with advanced disease vs. females with advanced disease (HR: 2.90, 1.60-5.32, P<.001; log-rank P<.001)**;** male patients with advanced disease vs. males with early disease (HR: 8.86, 2.58-30.49, P<.0001; log-rank P<.001); male patients with early disease vs. females with early disease (HR: 1.56, 0.47-5.18, P=.47; log-rank P=.47); female patients with advanced disease vs. males with early disease (HR: 3.06, 0.94-9.94, P=.06; log-rank P=.06); and female patients with advanced disease vs. females with early disease (HR: 4.77, 2.84-8.01, P<.0001; log-rank P<.0001).

Time measured in years following PBC diagnosis.

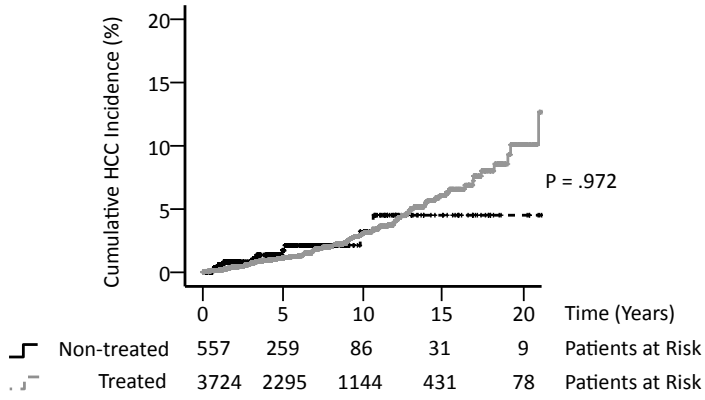


Female	85	59	52	47	40	34	28	Patients at Risk
Male	23	14	10	7	6	5	2	Patients at Risk

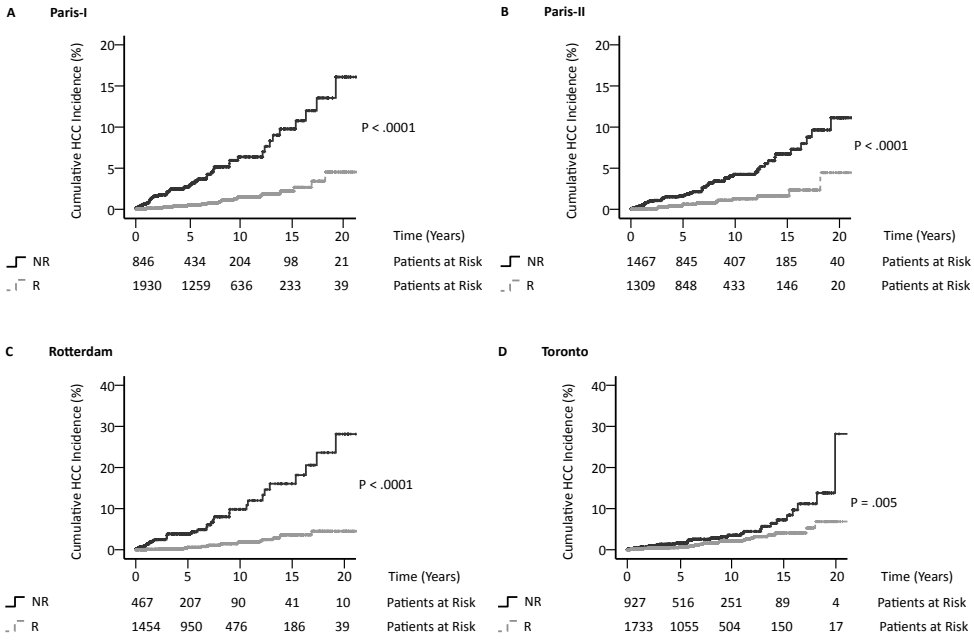
Supplementary Figure 2. Transplant-free survival according to gender in patients diagnosed with HCC

Kaplan Meier plot of subsequent transplant-free survival in men vs. women following development with HCC.

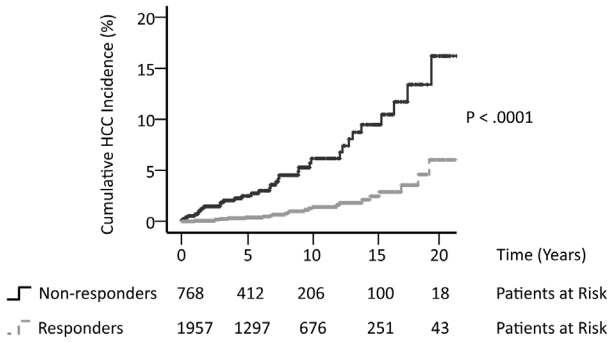
Time represented in months following tumor diagnosis.



Supplementary Figure 3. Cumulative HCC incidence stratified according to UDCA treatment
Kaplan Meier estimate of HCC incidence in UDCA treated versus untreated patients.
Time measured in years following calculation of biochemical response.



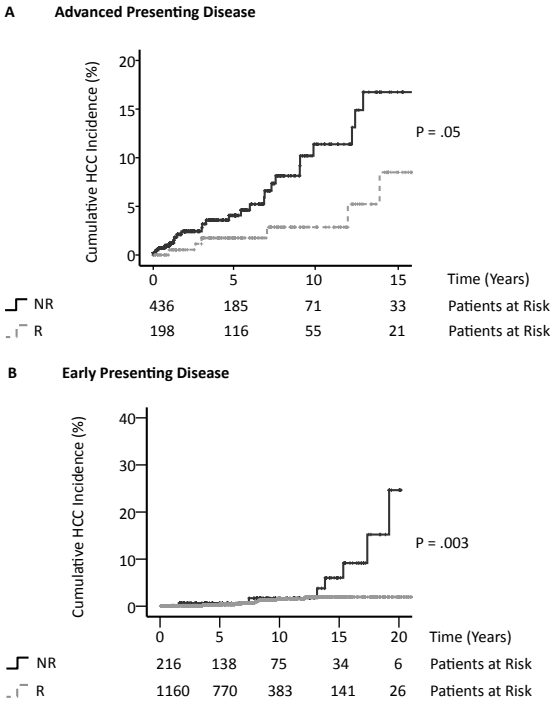
Supplementary Figure 4. Cumulative HCC appearance rates according to biochemical response criteria
Kaplan Meier estimates of HCC incidence in biochemical non-responders (NR) versus responders (R) as per the following criteria: (A) Paris I, 6.0 vs. 1.3 cases per-1,000 patient years; (B) Paris II: 4.4 vs. 1.1 cases per 1,000 patient years; (C) Rotterdam: 9.9 vs. 1.6 cases per 1,000 patient years; and (D) Toronto: 4.0 vs. 2.1 cases per 1,000 patient years. No significant difference in HCC incidence was observed through biochemical stratification via Barcelona criteria (data not presented).
Analysis pertains to UDCA-treated as well as untreated individuals in whom 12-month biochemical data was available to calculate response (24-months in the case of Toronto criteria). Time measured in years following calculation of biochemical response.



Supplementary Figure 5. Cumulative HCC appearance rates according to biochemical response assessed at 12-24 months

Kaplan Meier estimates of HCC incidence stratified according to biochemical response assessed at 12-24 months: 6.5 (non-responders) vs. 1.4 (responders) cases per-1,000 patient years.

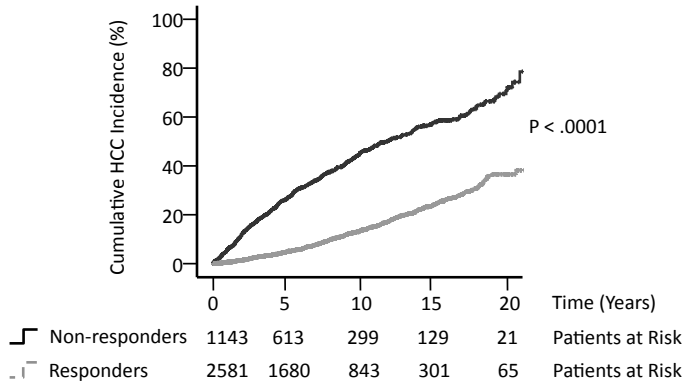
Analysis restricted to UDCA-treated patients only, in whom 12-24month biochemical data available to calculate response. Time measured in years following calculation of biochemical response.



Supplementary Figure 6. HCC incidence in patients with varying disease stage stratified according to biochemical response

Kaplan Meier estimate restricted to those with (A) advanced presenting disease and biochemical non-response (NR) versus response (R); 10.8 vs. 4.8 cases per 1,000 patient years; and (B) early presenting disease and biochemical non-response versus response: 4.0 vs. 1.0 cases per 1,000 patient years.

Analysis conducted in UDCA-treated and untreated patients in whom 12-month biochemical data available to calculate response. Time measured in years following calculation of biochemical response.



Supplementary Figure 7. Estimated HCC appearance rates according to biochemical response following multiple imputation

Kaplan Meier estimates of HCC incidence in biochemical non-responders versus responders (Paris-I) following multiple imputation: 59.6 vs. 15.4 cases per-1,000 patient years (HR: 3.74, 3.24-4.33; P<.0001).

Data presented for UDCA-treated patients only. Time zero from point of assessment of biochemical response.

SUPPLEMENTARY TABLES

Supplementary Table 1. Patient characteristics

	Total group (*n*=4565)	No HCC (*n*=4442)	HCC (*n*=123)
Median age time of diagnosis (IQR)	53.2 (45.1-62.1)	53.2 (45.1-62.1)	54.0 (47.3-62.1)
Males	468 (10%)	441 (10%)	27 (22%)
AMA positive*	4041 (89%)	3929 (89%)	112 (91%)
Not available	105 (2.3%)	100 (2.2%)	5 (4.1%)
Biochemical stage**			
Early	2020 (44%)	1993 (45%)	27 (22%)
Advanced	960 (21%)	908 (20%)	52 (42%)
Laboratory parameters (baseline)			
Albumin***	1.14 (1.06-1.23)	1.14 (1.06-1.23)	1.08 (1.00-1.19)
ALP***	2.10 (1.31-3.72)	2.10 (1.31-2.72)	2.23 (1.33-3.70)
ALT***	1.61 (1.00-2.58)	1.60 (1.00-2.57)	1.80 (1.21-2.90)
AST***	1.40 (0.92-2.20)	1.40 (0.92- 2.20	1.74 (1.21-2.82)
Bilirubin***	0.66 (0.45-1.06)	0.64 (0.45-1.00)	1.10 (0.80-2.18)
Platelets	240 (181-294)	242 (184-295)	182 (120-231)
Hepatic decompensation****	140 (3.1%)	122 (2.7%)	18 (15%)

* AMA status not available in 2.3% (*n*=105; total cohort), 2.2% (*n*=100; no HCC group) and 4.1% (*n*=5; HCC group) of patients.
** Rotterdam classification as per ter Borg *et al*. Baseline biochemical disease stage not calculable in 35% (*n*=1583; total cohort), 35% (*n*=1541; no-HCC group) and 34% (*n*=42; HCC group) of patients.
*** Given variations in assay techniques across the studied institutions, a ratio to the upper limit of normal (ULN) was taken.
**** Within 1st year of PBC diagnosis.

Supplementary Table 2. Baseline factors predictive of future HCC risk

	Univariate analysis		Multivariable analysis*	
	Hazard ratio (95% CI)	P-value	Adjusted hazard ratio* (95% CI)	P-value
Advancing age at PBC diagnosis**	1.21 (1.14-1.41)	.022	-	n.s.
Male gender	2.91 (1.90-4.48)	<.0001	5.41 (1.56-18.76)	.008
Higher serum AST	1.24 (1.13-1.36)	<.0001	-	n.s.
Advanced biochemical disease***	2.72 (1.43-5.18)	.022	2.71 (1.43-5.15)	.023
Thrombocytopenia****	1.65 (1.42-1.90)	<.0001	1.42 (1.11-1.92)	.013
Hepatic decompensation	9.89 (5.89-16.59)	<.0001	-	n.s.

* Adjusted for center-specific heterogeneity
** Per 10-year increase
*** As per to the criteria by ter Borg *et al.* Given that no significant difference in HCC incidence existed between those with moderate vs. late-stage disease, these two groups were combined in further analyses ('advanced biochemical disease').
**** Per-50 x10^3/mm^3 decline in platelet count

Supplementary Table 3. UDCA treatment exposure according to disease severity*

	Early disease	Advanced disease	Total	P-value
UDCA treated	1679	770	2449	
UDCA non-treated	309	182	491	.015
Total	1988	952	2940	
Years to UDCA treatment initiation (median; IQR)	0.3 (0.0-2.7)	0.4 (0.0-3.6)		.588

* Data presented for patients in whom > 12-month follow-up and baseline biochemical disease severity available.

Supplementary Table 4. HCC incidence in varying risk groups*

	Overall	**Male**	**Female**	**Advanced baseline disease**	**Early baseline disease**	**Biochemical non-response****	**Biochemical response****
Male	*6.7*	-	-	18.9	2.4	18.2	5.4
Female	*2.6*	-	-	6.9	1.5	5.2	1.1
Advanced baseline disease	*7.6*	18.9	6.9	-	-	11.2	4.4
Early baseline disease	*1.3*	2.4	1.5	-	-	4.7	1.2
Biochemical non-response**	*6.6*	18.2	5.2	18.2	4.7	-	-
Biochemical response**	*1.4*	5.4	1.1	5.4	1.2	-	-

* Expressed per 1,000-patient-years
** Paris-I; data presented for UDCA-treated patients only

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CHAPTER 6

Age, but not sex, alters response to ursodeoxycholic acid in patients with primary biliary cirrhosis – an international and multicenter study

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ABSTRACT

Background & Aim: Primary biliary cirrhosis (PBC) predominantly affects middle-aged women, whereas phenotypic and outcome data pertaining to men, and younger patients is limited. The aim of this study was to identify whether sex and/or age associate with distinct patterns of biochemical response and variation in transplant-free survival.

Methods: A longitudinal study was performed evaluating 4117 patients from the Global PBC Study cohort, with regards to biochemical response to ursodeoxycholic acid (UDCA) according to Paris-1 criteria and transplant-free survival. Both logistic regression and Cox regression analyses were performed, adjusted for age, sex, disease severity and center.

Results: Men were older at baseline (56.7±12.6 vs. 52.4±11.7, p<.0001), with more advanced disease as demonstrated by higher serum bilirubin, lower albumin and lower platelet count and exhibited lower biochemical response rates than women (Paris I criteria at 1 yr: 60% vs. 70%, p<.0001). On multivariable analysis, however, sex was not an independent predictor of response (OR 0.81, 95% CI, 0.62-1.07, p=.13) or transplant-free survival (HR 1.10, 95% CI 0.74-1.11, p=.34). By contrast, PBC patients with better biochemical profiles (serum bilirubin, alkaline phosphatase and AST levels) had a higher probability of response to UDCA (p<.0001). Younger patients had lower biochemical response rates than older individuals of matched disease stage, regardless of sex (OR 2.35, 95% CI 1.53-3.61, p<.0001).

Conclusion: Advanced baseline stage and age (but not male sex) are predictive of therapeutic failure and adverse clinical outcome in PBC. Increasing recognition of these phenotypic variants may contend for the earlier application of additional therapies prior to evaluation of 1-yr UDCA response.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic autoimmune disorder of cholestasis, in which patient outcome is largely dictated by development of cirrhosis and portal hypertension. Classical phenotypic descriptors imply disease restriction to middle-aged, often post-menopausal women with peak incidence between 40-60 years of age.[1-3] Thus, even large studies have provided limited insight into the influence of gender and age on disease outcomes.[4, 5]

Several studies have demonstrated that the clinical impact of PBC differs between the sexes and different age groups. Women are frequently symptomatic at presentation, with an increasing burden of pruritus,[6, 7] and scores higher in the fatigue domain of the PBC-40 quality of life questionnaire.[5] By contrast, male PBC patients more often present with advanced baseline disease,[5-7] harbor a greater risk of hepatocellular carcinoma (HCC)[8] and experience significantly poorer transplant-free survival.[4] Moreover, male sex has recently identified as an independent risk factor for incomplete response to ursodeoxycholic acid (UDCA), independent of presenting age, presence of portal hypertension, and biochemical indices of disease severity,[5] alluding to the possibility of a more rapidly progressive clinical course.

Age at baseline appears to add another layer of complexity to clinical phenotypes and the recent study conducted by the UK-PBC consortium not only recognized an increased prevalence of younger presenting women (25% aged 49 or less), but also an inverse correlation of patient age and likelihood of meeting biochemical response in females, but in males age appeared to have no significant impact on response.[5]

The aims of this study were to validate the negative prognostic impact of patient age and sex on treatment response and transplant-free survival using a large internationally representative and statistically powered cohort of PBC patients.

METHODS

Subjects and study design

This was a longitudinal retrospective study of treatment response and clinical outcomes in a well-defined cohort of adult PBC patients. Demographic, clinical and outcome data were collected from 15 centers across Europe and North America as part of the Global PBC Study Group database, as previously described.[9] The cohort included adult patients > 18 years of age diagnosed with PBC between 1959 and 2012, as defined by published criteria.[1, 10]

Time zero (baseline) was defined as the date of initiation of UDCA. The primary endpoints were biochemical response as per the Paris-I criteria (alkaline phosphatase [ALP] <3 times

the upper limit of normal (xULN), aspartate aminotransferase (AST) < 2xULN and normal serum bilirubin level),[11] generally considered as the criteria with best performance in predicting outcome,[5, 8] and liver transplant-free survival. Patients who did not meet clinical endpoints (liver transplant or death) were censored at their last date of available follow-up. This study was reviewed and approved by all local Institutional Review Boards across the 15 centers.

Statistical analysis

Continuous data are expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) depending on data distribution, and categorical data as proportions. Unpaired t-tests or the Mann-Whitney U-test were used to determine whether there were significant differences between groups for continuous variables, and differences in categorical data were analyzed using the Chi-square test. Unadjusted differences in survival between males and females were assessed using Kaplan-Meier analysis with comparisons made using the log-rank test. Univariable and multivariable associations were computed using logistic regression for biochemical response (odds' ratio, OR, and 95% confidence interval, CI) and Cox proportional hazards regression for transplant-free survival (hazards' ratio, HR, and 95% CI). Univariable analysis included: sex, age at diagnosis, era of diagnosis (before or after 1998, ie. the median date of diagnosis for the whole cohort), histologic stage as defined by Scheuer's[12] and Ludwig's criteria[2], biochemical stage as defined by the Rotterdam criteria[13] (early disease: normal bilirubin and albumin, moderately advanced: either abnormal bilirubin or albumin, advanced: abnormal bilirubin and albumin), biochemical response and surrogate of portal hypertension (platelet count < $100x10^3/mm^3$ or < $150x10^3/mm^3$).[14, 15] Age at baseline was analyzed as both a continuous variable and as a categorical variable grouped as < 35 and > 65 years and the intervening decades to allow for an equitable distribution for analysis.

The analyses were performed using multiple imputation by MCMC method for missing data (Proc MI in SAS version 9.3). Multiple imputation was based on the assumption that data were missing at random, with 10 imputed datasets created from iterations to reduce sampling variability. Rubin's rules were used for estimation of parameters of interest and standard error.[16, 17] The variables included in the process of imputation were: AST, alanine aminotransferase (ALT), ALP, total bilirubin, albumin and platelets.

Non-normally distributed data were transformed using log transformation. A two-sided P<.05 was considered statistically significant. Statistical analysis was performed using SPSS Software version 21.0 (SPSS Inc., Chicago, IL) and SAS version 9.3 (SAS Institute Inc., Cary, NC).

RESULTS

Patient characteristics

4845 patients were included in the Global PBC study group dataset; a full description of the cohort can be found in our previously published work.[8, 9] For the current study, a total of 4117 patients were analyzed after excluding those < 18 years of age and those with no or unknown treatment status. 413 (10%) were male and 3704 (90%) were female with a median follow-up of 7.7 years (IQR 3.9-12.0); 566 patients died and 320 were transplanted.

Clinical differences at start of follow-up between sexes and age groups

At baseline men were older (56.7 ± 12.6 years vs 52.4 ± 11.7 years, P<.0001), with lower serum albumin (1.12 times the lower limit of normal [xLLN] vs 1.14xLLN, P=.03), higher bilirubin (0.82xULN vs 0.62xULN, P<.0001) and were more likely to have thrombocytopenia (22% vs 14% with platelets <150x10^{3}/mm^{3}, P<.0001) in comparison with women (**Table 1**). Meanwhile, younger patients were more likely to be diagnosed at an earlier disease stage than older patients, with higher albumin and platelets (P<.0001) as well as lower bilirubin (P<.0004), though higher transaminases (P<.0001) (**Table 2**).

Effect of gender on biochemical response to UDCA

3978 (97%) of patients had at least 1 year of follow-up. Prior to adjusting for baseline factors, there was a significant difference in the crude biochemical response rate between males and females (60% vs 70%, P<.0001). However, after adjusting for parameters corresponding to disease severity (ie. platelet count, biochemical disease stage, baseline bilirubin and ALP), year of diagnosis and center, male sex was no longer an independent predictor of response (OR 0.81, 95%CI 0.62-1.07, P=.13) (**Table 3**). There were no clinically significant differences between bilirubin and albumin values for males and females within each biochemical disease stage (**Table 4**).

Table 1. Baseline Characteristics

Parameter	All patients N=4117	Male patients N=413	Female patients N=3704	P-value
Age at start of follow-up, mean ± SD	54.2 ± 11.8	57.8 ± 12.3	53.4 ± 11.7	< .0001
AMA positive, no. (%)	3631 (91)	379 (92)	3252 (90)	.19
Year of diagnosis, no. (%)				.68
<1990	811 (20)	811 (19)	732 (20)	
1990-2000	1633 (39)	1633 (38)	1478 (39)	
2000-2010	1515 (37)	1515 (39)	1352 (37)	
>2010	158 (4)	158 (4)	142 (4)	
Disease stage (biochemical),[b] no. (%)				< .001
Early	1686 (68)	133 (52)	1553 (70)	
Moderately advanced	598 (24)	85 (34)	513 (23)	
Advanced	192 (8)	36 (14)	156 (7)	
Disease stage (histological),[c] no. (%)				.84
Early stage disease (I or II)	1089 (68)	104 (68)	985 (68)	
Late stage disease (III or IV)	521 (32)	48 (32)	473 (32)	
Laboratory parameters[d]				
AST (xULN)	1.45 (0.94-2.23)	1.40 (0.92-2.13)	1.47 (0.94-2.25)	.37
ALT (xULN)	1.66 (1.03-2.60)	1.64 (1.03-2.68)	1.66 (1.03-2.60)	.89
ALP (xULN)	2.14 (1.36-3.79)	2.02 (1.40-3.49)	2.15 (1.35-3.80)	.39
Albumin (xLLN)	1.14 (1.06-1.23)	1.12 (1.03-1.23)	1.14 (1.06-1.23)	.03
Total bilirubin (xULN)	0.65 (0.46-1.00)	0.82 (0.59-1.44)	0.62 (0.44-1.00)	< .0001
Platelets, $x10^3/mm^3$	243 (186-297)	215 (160-261)	247 (189-301)	< .0001

Abbreviations: AMA, anti-mitochondrial antibody, SD, standard deviation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ULN, upper limit of normal; LLN, lower limit of normal.

[a]AMA status was unavailable for 112 patients (3 males and 109 females).

[b]Biochemical disease stage defined as per ter Borg et al.[15] (early: normal serum bilirubin and albumin levels, moderately advanced: abnormal bilirubin or albumin levels, advanced: abnormal serum bilirubin and albumin levels)

[c]Baseline biopsy was performed in 49% (2029 patients; 207 males and 1822 females). Baseline histological disease stage was unavailable in 21% (n=419, all patients), 27% (n=55, male patients) and 20% (n=364, female patients).

[d]Due to differences in normal thresholds between centers, laboratory values are listed as factors of the upper limit of lower limit of normal.

Table 2. Baseline characteristics according to age at follow-up start

Parameter	≤35 n=192	36-45 n=685	46-55 n=1228	56-65 n=1168	>65 n=844	P-value
Sex, no. (% male)	14 (7)	51 (7)	89 (7)	130 (11)	129 (15)	< .0001
AMA positive, no. (%)	166 (87)	595 (88)	1070 (88)	1033 (89)	767 (92)	.04
Diagnosis year, no. (%)						< .0001
<1990	28 (15)	109 (16)	237 (19)	269 (23)	168 (20)	
1990-1999	64 (33)	321 (47)	509 (41)	446 (38)	293 (35)	
2000-2010	94 (49)	234 (34)	441 (36)	403 (35)	343 (41)	
>2010	6 (3)	21 (3)	41 (3)	50 (4)	40 (5)	
Disease stage (biochemical)[a], no. (%)						.02
Early	69 (66)	231 (63)	488 (70)	522 (71)	376 (65)	
Moderately advanced	31 (30)	109 (30)	156 (23)	157 (21)	145 (25)	
Advanced	4 (4)	25 (7)	50 (7)	57 (8)	56 (10)	
Disease stage (histological)[b], no. (%)						.04
I or II	65 (76)	230 (69)	395 (70)	255 (64)	144 (63)	
III or IV	20 (24)	105 (31)	166 (30)	146 (36)	84 (36)	
Portal hypertension[c], no. (%)						
Platelets <100x10^3/mm^3	2 (1)	8 (1)	35 (3)	52 (5)	53 (6)	< .0001
Platelets <150x10^3/mm^3	7 (7)	28 (7)	78 (12)	115 (16)	132 (24)	< .0001
Laboratory parameters, median (IQR)						
AST (xULN)	1.69 (1.05-2.80)	1.90 (1.20-2.80)	1.56 (1.02-2.40)	1.31 (0.90-2.03)	1.23 (0.83-1.88)	< .0001
ALT (xULN)	2.25 (1.47-3.89)	2.50 (1.44-3.81)	1.90 (1.21-2.88)	1.50 (1.00-2.20)	1.20 (0.80-1.84)	< .0001
ALP (xULN)	2.27 (1.24-4.27)	2.58 (1.55-4.87)	2.37 (1.50-4.14)	2.06 (1.32-3.39)	1.81 (1.20-2.96)	< .0001
Albumin (xLLN)	1.17 (1.09-1.26)	1.16 (1.06-1.25)	1.16 (1.08-1.25)	1.14 (1.06-1.23)	1.11 (1.02-1.20)	< .0001
Total bilirubin (xULN)	0.62 (0.42-1.06)	0.71 (0.47-1.20)	0.60 (0.41-1.00)	0.67 (0.48-1.00)	0.67 (0.47-1.00)	.0004
Platelets (x10^3/mm^3)	263 (225-313)	277 (221-329)	252 (200-303)	233 (174-283)	215 (151-274)	< .0001

Abbreviations: SD, standard deviation; AMA, anti-mitochondrial antibody; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ULN, upper limit of normal; LLN, lower limit of normal

[a] Biochemical disease stage defined as per ter Borg et al.[15] (early: normal serum bilirubin and albumin levels, moderately advanced: abnormal serum bilirubin or albumin levels, advanced: abnormal serum bilirubin and albumin levels). Insufficient data for determination in 40% (88, 320, 534, 432, 267 for each age group).

[b] Baseline histological disease stage was not available in 23% (n=26), 21% (n=87), 15% (n=99), 25% (n=134), 24% (n=73) in each respective age group (listed from youngest to oldest).

[c] Platelet count was unavailable for 59% (85, 308, 550, 456, 300 for each respective age group listed from youngest to oldest).

Table 3. Multivariable logistic regression for factors affecting biochemical response to UDCA (Paris-I)

	Entire cohort (n=4117)		
Baseline variable	OR	95% CI	P-value
Male sex	0.81	0.62 - 1.07	.13
Older age (per decade)	1.10	1.10 - 1.22	< .001
Later era	1.02	1.01 - 1.03	< .0001
Platelet count (per 50x10^3/mm^3 decline)	0.94	0.89-0.98	.01
Early stage[a]	1.00		
Moderately advanced stage[a]	0.54	0.43-0.69	< .0001
Advanced stage[a]	0.46	0.31-0.67	< .0001
Baseline log bilirubin (xULN)	0.22	0.18 - 0.27	< .0001
Baseline log ALP (xULN)	0.37	0.32 - 0.42	< .0001

Abbreviations: ALP, alkaline phosphatase; OR, Odds' ratio; ULN, upper limit of normal.
[a] Biochemical disease stage defined as per ter Borg et al.[15] (early: normal serum bilirubin and albumin levels, moderately advanced: abnormal serum bilirubin or albumin levels, advanced: abnormal serum bilirubin and albumin levels).

Table 4. Median bilirubin and albumin levels stratified by biochemical stage

	Bilirubin xULN (IQR)	Bilirubin xULN (IQR)	P-value
Biochemical disease stage[a]			
Early	0.6 (0.5-0.8)	0.5 (0.4-0.7)	< .0001
Moderately advanced	1.2 (0.9-1.9)	1.1 (0.7-1.8)	.21
Advanced	2.3 (1.3-3.0)	1.9 (1.0-3.3)	.79
	Albumin xLLN (IQR	Albumin xLLN (IQR)	P-value
Biochemical disease stage[a]			
Early	1.2 (1.1-1.3)	1.2 (1.1-1.3)	.22
Moderately advanced	1.1 (1.0-1.2)	1.1 (1.0-1.2)	.63
Advanced	0.9 (0.8-1.1)	0.9 (0.8-1.0)	.49

Abbreviations: IQR, interquartile range; LLN, lower limit of normal; ULN, upper limit of normal.
[a] Biochemical disease stage defined as per ter Borg et al.[15] (early: normal serum bilirubin and albumin levels, moderately advanced: abnormal serum bilirubin or albumin levels, advanced: abnormal serum bilirubin and albumin levels).

Effect of age on biochemical response to UDCA

On univariable analysis, older age at diagnosis was associated with improved biochemical response (per-decade increase in age: OR 1.01, 95%CI 1.01-1.02, P<.0001). After adjusting for baseline factors associated with disease severity, older patients overall appeared to have significantly better response than younger patients according to the Paris-I criteria (**Table 3**). For each decade in age, there was an increase in response ranging from 10-50%: <35 years (reference), 36-45 years (OR 1.53, 95%CI 1.26-2.86, P=.05), 46-55 years (OR 1.90, 95%CI 1.26-2.86, P=.002), 56-65 years (OR 2.03, 95%CI 1.35-3.07, P=.001), >65 years (OR 2.35, 95%CI 1.53-3.61, P<.0001), (**Table 5**). There was no significant interaction between age and sex (P=.59).

Importantly, there was a strong interaction between age and bilirubin (P<.0001) and age and ALP levels (P=.0005); PBC patients with better biochemical profiles (serum bilirubin, ALP and AST levels) had a higher probability of response to UDCA (**Figure 1**). When categorizing patients according to their baseline biochemical profile older patients had a higher probability of response than younger patients (**Figure 1 A-C**), except for patients with extreme high baseline biochemical indices (**Figure 1 D**).

Table 5. Multivariable logistic regression evaluating the effect of age on biochemical response to UDCA (Paris-I)

	Entire cohort (n=4117)		
Baseline parameter	OR	95% CI	P-value
Male sex	1.22	0.93-1.59	.15
Later era	1.02	1.01-1.03	< .0001
Age at diagnosis (years)			
≤35	1.00		
36-45	1.53	1.00-2.35	.05
46-55	1.90	1.26-2.86	.002
56-65	2.03	1.35-3.07	.001
>65	2.35	1.53-3.61	< .0001
Platelet count (per $50x10^3/mm^3$ decline)	1.07	1.02-1.12	< .001
Early biochemical stage[a]	1.00		
Moderate biochemical stage	2.19	1.50-3.21	< .0001
Advanced biochemical stage	1.19	0.85-1.68	.30
Log bilirubin (xULN)	0.22	0.18-0.27	< .0001
Log ALP (xULN)	0.37	0.32-0.42	< .0001

Abbreviations: AST, aspartate aminotransferase; ULN, upper limit of normal; ALP, alkaline phosphatase

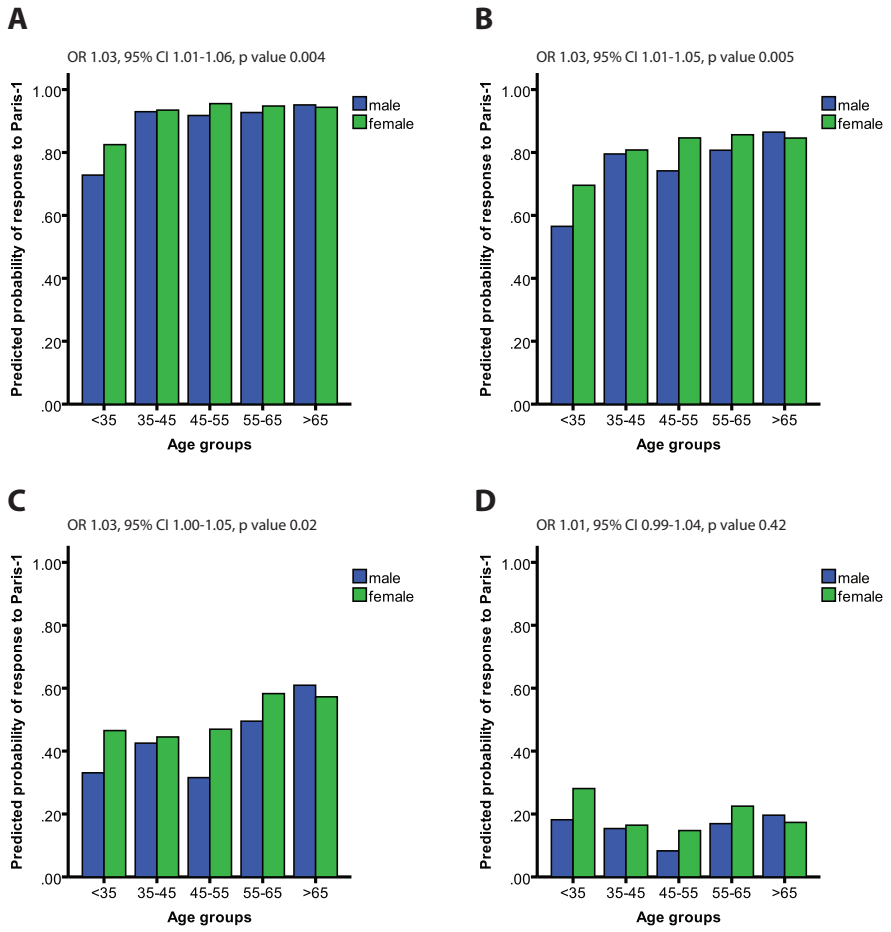

Figure 1. Predicted probability of response according to Paris-1 criteria age groups
Bar plots of the predicted probability of response according to Paris-1 criteria among age groups in (A) patients with a biochemical profile according to the 25th percentile of baseline serum alkaline phosphatase, bilirubin and AST levels (OR young vs. older patients 1.03, 95% CI 1.01-1.06, P = .0004), (B) according to the 50th percentile (OR young vs. older patients 1.03, 95% CI 1.01-1.05, P = .0005), (C) according to the 75th percentile (OR young vs. older patients 1.03, 95% CI 1.00-1.05, P = .02) and (D) according to the 50th percentile (OR young vs. older patients 1.03, 95% CI 0.99-1.04, P = .42). The dark bars represent the males and the light bars the females.

Effect of age and gender on transplant-free survival

On crude analysis of overall transplant-free survival, significantly more males died (20% vs 13%) or underwent liver transplant (11% vs 7%) (Log-rank P<.0001) (**Figure 2A**). However, after adjusting for age at start of treatment, year of diagnosis, biochemical disease stage, baseline bilirubin, baseline ALP and platelet count, the differences in survival between males and females was no longer significant (HR 1.10, 95%CI 0.74-1.11, P=.34) (**Figure 2B, Table 6**).

Table 6. Multivariable logistic regression for factors affecting survival

	Entire cohort (n=4117)		
Baseline variable	HR	95% CI	P-value
Males	1.10	0.74-1.11	.34
Older age (per decade)	1.54	1.43-1.64	< .0001
Later era	0.99	0.98-1.00	.21
Platelet count (per 50x10^3/mm^3 decline)	0.88	0.83-0.93	< .0001
Early biochemical stage	1.00		
Moderate biochemical stage	2.10	1.67-2.63	< .0001
Late biochemical stage	3.56	2.59-4.88	< .0001
Baseline log bilirubin (xULN)	1.95	1.73–2.19	< .0001
Baseline log ALP (xULN)	1.13	1.01–1.27	.04

Abbreviations: ULN, upper limit of normal; ALP, alkaline phosphatase.
[a] Biochemical disease stage defined as per ter Borg et al.[15] (early: normal serum bilirubin and albumin levels, moderately advanced: abnormal serum bilirubin or albumin levels, advanced: abnormal serum bilirubin and albumin levels).

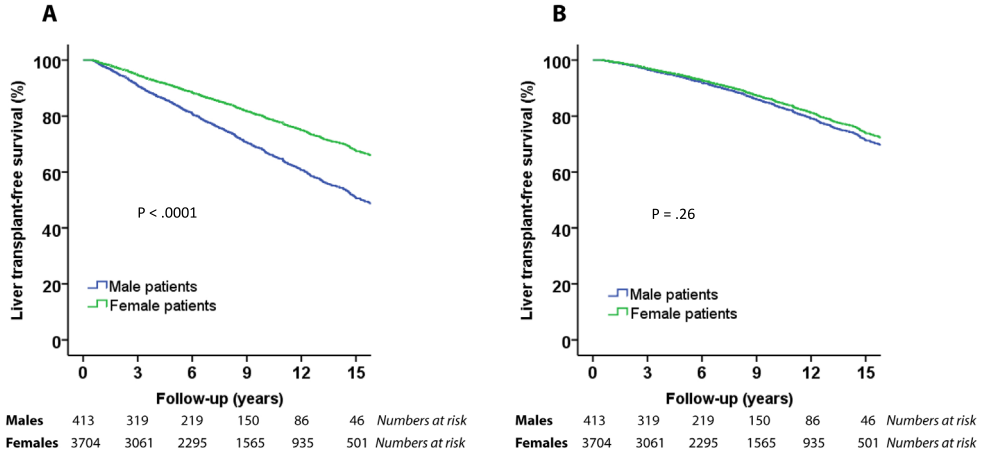

Figure 2. Crude and adjusted transplant-free survival curves between males & females
Survival curves showing (A) unadjusted (crude) survival and (B) adjusted survival between males and females with PBC. Differences in transplant-free survival between males and females were adjusted for age at start of treatment, year of diagnosis, biochemical disease stage, serum bilirubin levels, serum alkaline phosphatase levels and platelet count. Biochemical disease stage defined as per ter Borg et al.[15] (early: normal serum bilirubin and albumin levels, moderately advanced: abnormal serum bilirubin or albumin levels, advanced: abnormal serum bilirubin and albumin levels).

DISCUSSION

Similar to other diseases of autoimmune origin, the pathogenesis of PBC appears to be driven by fundamental differences in susceptibility across males and females, as well as across different age groups. The inherent challenges posed by the disease epidemiology of PBC has led to an elusive understanding of whether males or younger patients have a more aggressive disease phenotype. The cohort established by the Global PBC Group provides the opportunity to include subjects from both transplant and non-transplant centers from across Europe and North America. Owing to the size of the cohort and statistical validation through the use of center-specific stratification and multiple imputation, our study is strongly positioned to explore outcomes in small subgroups while minimizing bias. The results of our study demonstrate that sex does not appear to be an independent determinant of biochemical response or transplant-free survival, but rather that males present with more advanced disease, which has previously been associated with diminished treatment response and prognosis.[3, 8, 13] Younger patients are more likely to present with early disease, but have impaired biochemical response to UDCA compared with older patients, even after adjusting for disease severity. This effect was maintained across both male and female patients.

Earlier studies have demonstrated that male PBC patients generally present with more advanced disease, with higher rates of jaundice, variceal bleeding and thrombocytopenia[5, 6]. Asymptomatic males also presented at an older age than females, with a mean difference of approximately 5 years[5, 6]. Carbone et al. demonstrated in the UK-PBC cohort that male sex was an independent predictor of biochemical response (OR 0.90, 95% CI 0.83-0.97, P=.007) after adjusting for age, splenomegaly, creatinine, log bilirubin and log ALP. By contrast, our study demonstrated that male sex was not independently associated with biochemical response or with transplant-free survival. The lack of association between male sex and clinical outcomes in PBC suggests that sex is not an inherent determinant of treatment response or prognosis, but rather that male PBC patients are at greater risk of presenting with more advanced disease, with a greater degree of synthetic dysfunction and portal hypertension. A possible factor explaining this finding could be that the diagnosis of PBC is not sufficiently considered in males presenting with features of liver disease. However, this is highly speculative and it may well be that male PBC patients develop less frequent or less severe symptoms and therefore remain undiagnosed until later in the course of the disease.

Previous studies found that older patients appeared to have significantly better response than their younger counterparts[5] and that life expectancy of PBC patients diagnosed at 55 years or older is comparable with a matched population.[18] In particular, Carbone et al. found that when results were stratified by sex, it appeared that older female patients have significantly better response than younger females, but that males had similar response

across all age groups. Our data demonstrated instead that the differences in response across age groups were associated with differences in disease stage, rather than sex. This difference in results may have in part be due to the smaller number of male patients in each age category in the UK cohort, with less than 10 patients in the under 40 and above 70 year age categories and less than 40 patients in the remaining age categories. Thus, it is possible that patients with early disease may not have been as well represented, particularly in males, given their propensity for presenting with advanced disease. Notably, while response appeared to increase with age, our study did not reveal a clear age threshold below which there was a significant reduction in biochemical response.

There are several potential reasons for diminished biochemical response in younger patients. One possibility is that younger patients may have reduced compliance. Disparities in response may also be related to underlying disease pathology. Patients with ductopenia have been previously demonstrated to have diminished response to UDCA[19], and descriptions of a severe ductopenic variant of PBC all involved patients younger than 50 years of age,[20] Thus, it is possible that younger patients who present with early PBC have a predominantly ductopenic phenotype which is particularly resistant to UDCA treatment, but whose symptoms or cholestatic biochemistry lead to a diagnosis early in the disease course. Additionally, in our cohort, patients under the age of 45 appeared to have higher AST and ALT, suggesting more exuberant histologic inflammation. Interestingly, Carbone et al. found that younger patients were more likely fail therapy based on transaminase criteria,[5] which collectively implies a more hepatitic phenotype.

In conclusion, age, irrespective of sex, has the greatest impact on biochemical response and transplant-free survival. Our data suggests that males appear to be diagnosed at more advanced stages of disease rather than that they exhibit a more aggressive phenotype. It is thus important to prevent diagnostic delays by maintaining a high index of suspicion for PBC in male patients and aggressively managing any potential concomitant causes of progressive fibrosis. Additionally, young patients with early disease should be monitored carefully, with early consideration for additional therapies, as they appear to be at greatest risk of incomplete biochemical response to UDCA. Further studies are required to unravel the mechanisms underlying the diminished treatment response to UDCA in young patients.

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CHAPTER 7

Summary and discussion

BACKGROUND

Chapter 1 provides a general overview of primary biliary cirrhosis (PBC) and summarizes current knowledge regarding the prediction of complications and long-term outcomes. PBC, occurring in 1 in 1000 women over the age of 40,[1] is a prototype vanishing bile duct disorder, characterized by a chronic non-suppurative destructive cholangitis and usually a slowly progressive course.[2] The reported incidence and prevalence varies worldwide; a recent Dutch study found an incidence of 1.1 per 100,000 and a prevalence of 13.2 per 100,000 Dutch inhabitants.[3] The disease may ultimately lead to severe bile duct loss (ductopenia) accompanied by increasing fibrosis, finally resulting in cirrhosis, liver failure and premature death. Liver transplantation has a significant beneficial effect on outcome in patients who progress to end-stage disease, however this ultimate therapeutic option is only available for a selected group of patients. Ursodeoxycholic acid (UDCA) is the only established medical treatment over the past 25 years.[4, 5] It is generally accepted that UDCA improves prognosis in the majority of cases. However, the response to treatment is insufficient in a subset of patients who still have an unmet medical need for effective additional therapy. Timely and reliable assessment of the response to therapy and prediction of outcome is of key importance in clinical management and an essential requirement in patient counseling and timing of diagnostic and therapeutic interventions.

SURROGATE ENDPOINTS IN PRIMARY BILIARY CIRRHOSIS

At present there is considerable interest from hepatologists and the pharmaceutical industry to expand the available medical treatment options for PBC. The efficacy of a number of new drugs has been explored during the last decade including budesonide,[6-8] anti-retroviral therapy,[9] rituximab,[10] beza- and fenofibrates[11] and obeticholic acid.[12] The ultimate goal of treatment is to cure or completely inactivate the disease and thereby prevent the development of liver cirrhosis and liver failure. Other equally important goals are to improve quality of life and effectively deal with invalidating symptoms, such as fatigue and pruritus. Ideally, in clinical trials evaluating disease modifying therapies the effect of treatment on hard clinical endpoints, such as complications, liver transplantation or death, should be evaluated.[13] However, PBC runs a slowly progressive course over many years or even decades. Evaluation of the efficacy of therapeutic interventions on hard clinical outcomes is therefore highly problematic as this requires unrealistic, long-term studies including large number of patients, especially when the study aims to modify early disease. This challenge can be clearly illustrated by the results of UDCA trials performed in the early nineties;[14, 15] although significant improvements of laboratory variables, such as alkaline phosphatase

and bilirubin, were shown in UDCA-treated patients within a follow-up period of 1 or 2 years, many studies were unable to document a beneficial effect on the natural course of the disease. Thus, there is an urgent need of reliable surrogate endpoints of outcome to predict the effect of an intervention on clinical outcome in an early phase of treatment.

Biochemical variables are of particular interest as surrogate endpoints, since they are readily available, objective and cheap to measure. Serum bilirubin is well established as an independent predictor of prognosis in PBC.[16, 17] However, serum bilirubin levels will change relatively late in the course of disease, implying that changes in this variable will not provide useful information in patients with non-advanced disease and normal levels. Alkaline phosphatase, a well-established marker of cholestasis and of key importance from a diagnostic point of view, may potentially be a more broadly applicable surrogate endpoint in PBC. **Chapter 2.1** contains a study that aimed to determine to what extent serum alkaline phosphatase and bilirubin levels individually and in combination, correlate with transplant-free survival and whether these variables can be regarded as robust surrogate endpoints in therapeutic PBC trials. We analysed an unprecedented large international database of patients from European and North-American centers and we were able to show that both increased serum alkaline phosphatase and bilirubin levels were strongly associated with reduced transplant-free survival in patients with PBC and that a combination of both variables improved their prognostic significance. These associations were independent of the use of UDCA and follow-up time and held for multiple subgroups. These data support that both alkaline phosphatase and bilirubin provide meaningful surrogate endpoints in PBC that can reasonably be used in clinical trials. There is a high unmet medical need for new therapies for this rare autoimmune liver disease, and this study provides an important impetus for the selection of appropriate endpoints and to facilitate the conduct of meaningful therapeutic intervention trials in the absence of long-term outcome studies.

Chapter 2.1 also discusses optimal thresholds for alkaline phosphatase and bilirubin levels to discriminate high- and low-risk patients that may be used as clinical trial surrogate endpoints. For alkaline phosphatase this was 2.0 times the upper limit of normal (ULN) after one year UDCA therapy, although other thresholds between 1.5 and 3.0 times the ULN may work equally well. Further, a percentage decrease of alkaline phosphatase or bilirubin levels after one year UDCA therapy from baseline was not superior in comparison to absolute levels after one year therapy in predicting transplant-free survival. For bilirubin levels 1.0 time the ULN appeared to be the optimal threshold. Based on current study, we were not able to recommend specific thresholds for a clinical trial in general, since the designation of clinical trials implies the a priori requirement to estimate the quantitative effect of an experimental intervention on a given endpoint.

In **chapter 2.2** we showed that bilirubin and alkaline phosphatase have not been fully validated as true surrogate endpoints with the highest level of evidence. According to strict

and difficult to meet criteria, relatively few really validated surrogate endpoints have been established.[18] It has been suggested that the best way to evaluate the utility of a biomarker as a good surrogate endpoint, might be a meta-analysis of clinical trials of one or more interventions.[18] Unfortunately, this is not feasible in a disease as PBC with only one licensed therapy. Our approach was therefore to conduct a rigorous patient-level meta-analysis using data collected from existing cohorts of patients at centers across North America and Europe with long-term follow-up data of large numbers of PBC patients. This design has sufficient power to intensively study alkaline phosphatase and bilirubin as potential surrogate endpoints in different settings, sub-populations and at different time points. Based on our current results we postulate that alkaline phosphatase and bilirubin are "reasonably likely to predict clinical benefit" in PBC,[19] of relevance to future trial design for new therapeutic agents. Future studies should be performed in PBC patients treated with new agents to increase the levels of evidence for alkaline phosphatase and bilirubin levels as surrogate endpoints.

RISK STRATIFICATION OF OUTCOMES IN PRIMARY BILIARY CIRRHOSIS

Liver transplantation or death

UDCA is not an uniformly effective drug and the results of several studies indicate that the prognosis of patients insufficiently responding to treatment is markedly worse compared with the general population.[20-24] These studies used quantitative biochemical changes (response criteria) to classify patients as either responders or non-responders to UDCA. Despite the clear relevance of biochemical response it has not been established whether biochemical response to UDCA is considered an important tool in clinical practice and whether it is used to guide further decision-making, in particular with respect to possible additional second-line treatment. **Chapter 3** reports a retrospective study assessing the impact of biochemical response to UDCA regarding management decisions in a large, nationwide cohort of PBC patients. We found that for the majority of UDCA non-responders medical management was not changed.

Obviously, the results of this study should be interpreted with great caution. In particular, it must be recognized that the majority of included patients were treated with UDCA well before emergence of the concept of biochemical response and that, in the context of this study, this response was assessed retrospectively. Therefore, by definition, decisions with respect to patient management could not have been influenced by assessing treatment response with one of the currently available tools. Irrespective of a formal response evaluation, however, our data suggest that management was modified in only a minority of cases despite persistently, occasionally markedly, abnormal biochemical liver tests.

Moreover, our data demonstrate that during the last decade, despite increased awareness of the importance of sufficient biochemical improvement upon treatment with UDCA, this did not translate yet into an increase in response-guided management.

Another major factor that must be stressed when interpreting the results of this study is the lack of evidence-based alternative treatments for PBC until now. This may largely explain why potentially effective drugs, including budesonide and fibrates, were rarely used. During recent years, evidence is accumulating that fibrates may have an additional, beneficial effect in UDCA-treated PBC.[25-29] The same applies to budesonide[6-8] and obeticholic acid,[12] drugs that are currently undergoing randomized controlled trial evaluation. It seems likely that within a few years the therapeutic landscape for PBC will have changed considerably and an evidence-based response guided treatment in PBC will be reality, potentially with a number of second-line treatment options available. To further study and assess the relevance of biochemical response criteria in current clinical practice and to overcome limitations of current study a cross-sectional study may be performed.

Further, this study emphasizes the importance of adequate UDCA dosage. About 40% of UDCA non-responders in whom the dosage was increased showed laboratory improvements allowing them to be reclassified as responders. Indeed, a multivariable analysis of factors predictive of response confirmed that higher UDCA dosage per kilogram was an independent predictor of response. These findings are in line with previous studies showing that UDCA doses in the range of 13-15 mg/kg/day are more effective than lower doses.[30, 31] Therefore, adequate dosing of UDCA remains of crucial importance.

Stratification tools, based on biochemical liver tests results after one or two years of UDCA exposure, such as the Barcelona, Paris I and II, Toronto and Rotterdam criteria, have all been shown useful to identify patients with or without sufficient treatment response.[20-24] However, these criteria were all based on dichotomized variables, potentially leading to loss of important predictive information. Further, comparison of these tools resulted in considerable differences in risk stratification.[32] Not less important, none of the models included age as established key prognostic factor. In **chapter 4,** we developed and validated a new unifying score (GLOBE score) with optimal ability to identify UDCA-treated patients with an insufficient treatment effect, based on the readily obtainable, biochemical and clinical variables age, bilirubin, alkaline phosphatase, albumin and platelet count. The prognostic performance of this score (C statistic 0.81, 95% CI 0.79-0.83) was markedly better than that of previously proposed criteria. Whilst the GLOBE score accurately predicts high risk, equally it predicts low risk, allowing development of care pathways that de-escalate follow-up back to primary care in appropriately stratified low risk patients. This is as important as high risk identification, in an era where cost-efficacy of new therapies is paramount. We believe that the GLOBE score represents a major step forward in the management of patients with PBC. The score is readily calculated, using a web application (www.globalpbc.com).

Hepatocellular carcinoma (HCC)

Life expectancy of PBC patients is largely determined by development of cirrhosis, portal hypertension and HCC.[4, 33, 34] HCC is the fifth most common cause of cancer and the third most common cause of cancer-related death[35] and usually arises in patients with underlying chronic liver disease. Across the spectrum of liver disease the incidence of HCC appears greatest among individuals with advanced fibrosis/cirrhosis, particularly men.[36] However, such observations in PBC frequently represent the results of single centre studies of limited size, or are not immediately generalisable to Western practice.[37-47] To address and overcome limitations to current knowledge we described in **chapter 5** the incidence of HCC, and risk factors for development of this tumour across a large, and internationally representative population. In doing so we confirmed that HCC is a critical event in the clinical course of PBC and is associated with a poor outcome.

In agreement with previous studies male sex and advanced biochemical disease were found to be major risk factors. Although the incidence was significantly greater in male patients and those with advanced disease, on multivariable analysis only thrombocytopenia and biochemical non-response retained statistical significance and superseded the effect of gender and other tested parameters of disease stage. Risk was significantly greater in biochemical non-responders and this also applied to patients who fulfilled criteria for non-response but never received UDCA. It is likely therefore that achievement of biochemical response according to specific criteria—irrespective of whether this occurs in the context of therapy—infers a surrogate associated with improved HCC-free survival rather than a chemo-preventative effect of UDCA.

The emergence of well-substantiated treatment response criteria has allowed accurate prediction of transplant-free survival in patients with PBC,[20-24, 48] and herein we illustrated that failure to achieve the same biochemical endpoints confers increased HCC risk. The low risk for HCC among biochemical non-responders, even in men and individuals with evidence of advanced disease at presentation, questions routine HCC surveillance in well-treated patients irrespective of gender and disease stage. Therefore, we recommend particular attention to: (1) male patients who either fail to achieve biochemical response (irrespective of disease stage), or in whom cirrhosis is already established (irrespective of biochemical response status); and (2) all female non-responders with evidence of advanced disease. Herein our globally representative data add new knowledge to HCC risk in PBC and informs ongoing discussions about stratified treatment and surveillance. We recommend that future studies should investigate the role of the earlier proposed GLOBE score with respect to stratification of HCC risk.

THE IMPACT OF AGE AND SEX ON OUTCOMES IN PRIMARY BILIARY CIRRHOSIS

The epidemiology of PBC poses inherent challenges in understanding the natural history of the disease in different subgroups. About 90% of patients with PBC are women, with the highest incidence occurring between 40-60 years of age, thus even large studies have provided limited insight into the influence of gender and age on disease outcomes.[49, 50] Several studies have demonstrated that the clinical impact of PBC differs between the sexes and different age groups. Women are frequently symptomatic at presentation, with an increasing burden of pruritus,[51, 52] and scores higher in the fatigue domain of the PBC-40 quality of life questionnaire.[53] By contrast, male PBC patients more often present with advanced baseline disease,[51-53] harbor a greater risk of hepatocellular carcinoma (HCC)[54] and experience significantly poorer transplant-free survival.[50] Moreover, male sex has recently identified as an independent risk factor for incomplete response to ursodeoxycholic acid (UDCA), independent of presenting age, presence of portal hypertension, and biochemical indices of disease severity,[53] alluding to the possibility of a more rapidly progressive clinical course.

Age at baseline appears to add another layer of complexity to clinical phenotypes and the recent study conducted by the UK-PBC consortium not only recognized an increased prevalence of younger presenting women (25% aged 49 or less), but also an inverse correlation of patient age and likelihood of meeting biochemical response in females, but in males age appeared to have no significant impact on response.[53] **Chapter 6** shows the results of a study addressing the question whether younger age or male sex are independently associated with diminished biochemical response and impaired transplant-free survival. We showed that sex does not appear to be an independent determinant of biochemical response or transplant-free survival, but rather that males present with more advanced disease, a condition that has previously been associated with diminished treatment response and prognosis.[22, 55] Further, our data demonstrated that younger patients respond less favorably to treatment and that the differences in response across age groups were associated with differences in disease stage, rather than sex. This is in contrast with the finding of Carbone et al. who showed that older female patients respond significantly better than younger females, while no effect of age on treatment response was apparent in males. This difference in results may have in part be due to the smaller number of male patients in each age category in the UK cohort. Thus, it is possible that patients with early disease may not have been as well represented, particularly in males, given their propensity for presenting with advanced disease.

There are several potential reasons for diminished biochemical response in younger patients. One possibility is that younger patients may have reduced compliance. Disparities

in response may also be related to underlying disease pathology. Patients with ductopenia have been previously demonstrated to have diminished response to UDCA[23], and descriptions of a severe ductopenic variant of PBC all involved patients younger than 50 years of age,[56] Thus, it is possible that younger patients who present with early PBC have a predominantly ductopenic phenotype which is particularly resistant to UDCA treatment, but whose symptoms or cholestatic biochemistry lead to a diagnosis early in the disease course. Additionally, in our cohort, patients under the age of 45 appeared to have higher AST and ALT, suggesting more exuberant histologic inflammation. Interestingly, Carbone et al. found that younger patients were more likely fail therapy based on transaminase criteria,[53] which collectively implies a more hepatitic phenotype.

Thus, age, irrespective of sex, has the greatest impact on biochemical response and transplant-free survival. It is thus important to prevent diagnostic delays by maintaining a high index of suspicion for PBC in male patients and aggressively managing any potential concomitant causes of progressive fibrosis. Additionally, young patients with early disease should be monitored carefully, with early consideration for additional therapies, as they appear to be at greatest risk of incomplete biochemical response to UDCA. Further studies are required to unravel the mechanisms underlying the diminished treatment response to UDCA in young patients.

CONCLUSIONS AND FUTURE PERSPECTIVES

With new promising drugs under evaluation risk stratification of patients at high- or low-risk of outcomes is of critical importance in UDCA-treated PBC at an early phase of the disease course. Biochemical variables, such as bilirubin and alkaline phosphatase, are attractive in this regard and can reasonably be used as surrogate endpoints of clinical endpoints, such as liver transplantation and death. To increase the levels of evidence for alkaline phosphatase and bilirubin levels as surrogate endpoints and to support its generalizability, additional studies should be performed in PBC patients treated with new agents. Currently, a risk score (GLOBE score), which comprises the readily available variables age, bilirubin, alkaline phosphatase, albumin and platelet count, could be an easy to use clinical tool to select UDCA-treated PBC patients in need of additional therapies.

With respect to liver cancer development in PBC, there is a relatively low incidence of HCC development among those who achieved adequate biochemical response, even in men and individuals with evidence of advanced presenting disease. This questions routine HCC surveillance in well-treated patients irrespective of gender and disease stage. Future studies should investigate the role of the GLOBE score with respect to stratification of HCC risk.

Sex does not appear to be an independent determinant of biochemical response or transplant-free survival. Particular awareness should be given to young patients with early PBC who do not respond well to UDCA. It seems likely that these patients have the potential to benefit more from additional PBC therapies than older patients. Further studies are required to unravel the mechanisms underlying the diminished treatment response to UDCA in young patients.

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CHAPTER 8 - APPENDICES

NEDERLANDSE SAMENVATTING

ACHTERGROND

In **hoofdstuk 1** wordt een algemeen overzicht gegeven van de ziekte primaire biliaire cirrose (PBC). Er wordt dieper ingegaan op de prognose van de aandoening en het voorspellen van de noodzaak tot een levertransplantatie en van sterfte.

PBC is een auto-immuunziekte van lever met een kenmerkend langzaam progressief beloop. Een chronische non-suppuratieve ontsteking van de kleine galwegen kan leiden tot verlies van de galgangen (ductopenie), het ontstaan van leverfibrose, oftewel verlittekening van de lever, en uiteindelijk levercirrose.[1] Cirrose wordt over het algemeen beschouwd als het eindstadium van de leverziekte waarbij er leverfalen kan optreden waaraan patiënten vroegtijdig kunnen overlijden. Levertransplantatie is een goede therapeutische optie bij vergevorderde ziekte die echter alleen kan worden toegepast bij een selecte groep van patiënten. Verder gaat levertransplantatie gepaard met belangrijke morbiditeit en mortaliteit en terugkeer van de ziekte in de nieuwe lever is mogelijk.

De ziekte is relatief zeldzaam en komt met name voor bij vrouwen van middelbare leeftijd. In Nederland wordt er elk jaar 1 persoon per 100.000 Nederlandse inwoners gediagnosticeerd met de ziekte PBC (incidentie) en er zijn op dit moment ongeveer 13 personen met PBC per 100.000 inwoners (prevalentie).[2] Wereldwijd worden uiteenlopende incidentie- en prevalentiecijfers beschreven;[3] gemiddeld genomen komt PBC voor bij 1 op de 1000 vrouwen boven de 40 jaar.

Er is op dit moment slechts één officieel geregistreerde medicamenteuze behandeling, namelijk ursodeoxycholzuur (UDCA).[4, 5] UDCA kan de ziekte niet genezen, maar kan het ziekteproces wel vertragen, waardoor er een positief effect is op de levensverwachting van PBC patiënten. Een deel van de met UDCA behandelde patiënten heeft een normale levensverwachting. Helaas hebben sommige patiënten die met UDCA behandeld worden toch een progressief ziektebeloop en deze patiënten zouden gebaat kunnen zijn bij nieuwe therapieën die op dit moment worden onderzocht. Het tijdig bepalen van de respons op UDCA behandeling en het vroegtijdig kunnen voorspellen welke patiënten een hoog risico hebben op het ontwikkelen van leverfalen zijn van kritisch belang voor een adequate behandeling van PBC patiënten.

SURROGAAT EINDPUNTEN IN PRIMAIRE BILIAIRE CIRROSE

De afgelopen jaren zijn diverse geneesmiddelen onderzocht op hun effectiviteit, zoals budesonide,[6-8] retuximab,[9] antivirale middelen,[10] bezafibraat, fenofibraat[11] en obeticholzuur.[12] Het belangrijkste doel van nieuwe therapieën is om de ziekte volledig te genezen dan wel om het ziekteproces te inactiveren, zodat de ontwikkeling van levercirrose en leverfalen kan worden voorkomen. Een ander belangrijk doel is om de kwaliteit van leven van PBC patiënten te verbeteren, met name door het behandelen van invaliderende symptomen, zoals jeuk en moeheid.

Idealiter worden in klinische studies harde klinische uitkomstmaten gebruikt, zoals leverfalen of overlijden,[13] echter door het langzaam progressieve beloop van PBC treden deze uitkomstmaten vaak pas na jaren op. Om klinisch relevante verschillen aan te tonen in uitkomstmaten tussen patiënten die behandeld worden met een nieuw geneesmiddel of de standaardbehandeling krijgen moeten langdurige studies worden verricht, met grote aantallen patiënten. De klinische studies uit de jaren negentig naar de werkzaamheid van UDCA zijn illustratief voor dit probleem.[14, 15] Ondanks dat er in diverse studies een significante verbetering werd aangetoond van serum levertesten na 1 of 2 jaar behandeling met UDCA, konden er geen significante verschillen worden aangetoond in de transplantatievrije overleving. Een latere meta-analyse van individuele patiëntengegevens van meerdere gecontroleerde trials, waarbij patiënten tot 4 jaar werden vervolgd, was wel in staat om een overlevingswinst aan te tonen. In PBC is er dus een grote behoefte aan surrogaat eindpunten; eindpunten die al vroeg in het beloop van de ziekte het risico op harde klinische eindpunten kunnen voorspellen.

Biochemische variabelen zijn interessant als potentiële surrogaat eindpunten, aangezien ze direct beschikbaar, objectief, niet-invasief en goedkoop zijn. Veel studies hebben laten zien dat serum bilirubine een onafhankelijke prognostische marker is in PBC;[16, 17] hogere bilirubinewaarden zijn geassocieerd met een kortere transplantatievrije overleving. Echter, serum bilirubinewaarden stijgen pas relatief laat in het ziekteproces, waardoor bilirubine minder aantrekkelijk is als surrogaat eindpunt. Het serum alkalische fosfatase lijkt daarentegen een interessantere surrogaat marker, aangezien dit enzym indicatief is voor de mate van cholestase en de waarden karakteristiek verhoogd zijn in alle ziektestadia. In **hoofdstuk 2.1** hebben we onderzocht of alkalische fosfastase en bilirubine waarden, alleen of in combinatie, geassocieerd zijn met transplantatievrije overleving en of deze bloedtesten gebruikt kunnen worden als robuuste surrogaat eindpunten in therapeutische trials. Hiervoor hebben we een grote internationale PBC database, the Global PBC Study Group database, geanalyseerd met langetermijngegevens van PBC patiënten uit diverse Europese en Amerikaanse ziekenhuizen. We waren in staat om aan te tonen dat hogere alkalische fosfatase en bilirubine waarden sterk geassocieerd zijn met een verminderde

transplantatievrije overleving en dat de combinatie van beide variabelen het voorspellend vermogen verbetert. Deze associatie werd gevonden op verschillende tijdstippen, in verschillende subgroepen en bij zowel UDCA behandelde als onbehandelde patiënten. Hiermee werd aannemelijk gemaakt dat alkalische fosfatase en bilirubine waarden kunnen worden gebruikt in klinische studies als surrogaat eindpunten voor harde klinische uitkomstmaten, zoals levertransplantatie of overlijden.

Daarnaast hebben we in **hoofdstuk 2.1** afkapwaarden voor alkalische fosfatase en bilirubine waarden bepaald, waarmee hoog- en laagrisico patiënten het beste kunnen worden onderscheiden. Voor alkalische fosfatase bleek dit afkappunt 2.0x de bovenste normaalwaarde te zijn, echter andere afkapwaarden tussen de 1.5-3.0x de bovenste normaalwaarde bleken even effectief te zijn. Voor bilirubine bleek 1.0x de bovenste normaalwaarde het optimale afkappunt te zijn.

In **hoofdstuk 2.2** gaan we in op een commentaar van Giljaca et al.[18] die stellen dat er onvoldoende bewijs geleverd is om alkalische fosfatase en bilirubine te beschouwen als valide surrogaat eindpunten. In de literatuur wordt gesuggereerd dat het beste bewijs geleverd kan worden door een meta-analyse te verrichten van klinische trials van het liefst zoveel mogelijk onderzochte interventies.[19] Helaas is dit niet mogelijk bij een ziekte zoals PBC, aangezien er slechts één geregistreerde behandeling is. Strikt genomen zijn er van de surrogaat eindpunten die in de kliniek worden gebruikt maar weinig die op deze manier gevalideerd zijn. Door gebruikt te maken van een groot, internationaal en multicenter cohort met langetermijngegevens van individuele PBC patiënten hebben we een uitgebreide meta-analyse kunnen verrichten, waarin we onder diverse omstandigheden en in multipele subgroepen, waaronder UDCA behandelde en onbehandelde patiënten, hebben laten zien dat alkalische fosfastase en bilirubine waarden geassocieerd zijn met levertransplantatievrije overleving. Ondanks dat niet aan het hoogste level van bewijs kan worden voldaan, suggereren onze resultaten dat alkalische fosfatase en bilirubine redelijkerwijs gebruikt kunnen worden als eindpunten in klinische studies.[20] Om het bewijs te vergroten voor alkalische fosfatase en bilirubine waarden als surrogaat eindpunten zouden toekomstige studies moeten worden verricht in PBC patiënten die worden behandeld met nieuwe geneesmiddelen.

RISICO STRATIFICATIE IN PRIMAIRE BILIAIRE CIRROSE

Lever transplantatie of overlijden

Helaas blijkt dat in een deel van de PBC patiënten de resultaten van behandeling met UDCA suboptimaal zijn. Diverse studies hebben respons criteria beschreven, bestaande uit bloedtesten, om patiënten na 1 of 2 jaar behandeling te kunnen classificeren als responder of non-responder.[21-25] Ondanks dat deze criteria gevalideerd zijn, is het

onduidelijk of ze ook daadwerkelijk gebruikt worden in de praktijk om beslissingen te nemen aangaande de behandeling van PBC patiënten. In **hoofdstuk 3** presenteren we de resultaten van een retrospectief verrichtte Nederlandse studie waarin onderzocht werd welke behandelbeslissingen er genomen zijn bij UDCA non-responders. Als belangrijkste bevinding vonden wij dat er in het merendeel van de gevallen geen veranderingen van het (medicamenteuze) beleid volgden bij onvoldoende respons op behandeling.

Deze studie moet met de nodige voorzichtigheid worden geïnterpreteerd. In deze studie werden patiënten in retrospectie ingedeeld in responders en non-responders. Een groot deel van de patiënten werd echter reeds behandeld met UDCA voordat het concept van biochemische respons ingang vond en voordat respons criteria werden gepubliceerd in de literatuur. Voor dat deel van de patiënten konden behandelbeslissingen uiteraard niet worden beïnvloed door de berekende respons criteria. Echter, onze studie toont wel aan dat ondanks dat er nog geen formele biochemische respons criteria beschikbaar waren, er nauwelijks aanpassingen aan de behandeling werden gemaakt, ook niet bij patiënten met persisterende en soms sterk abnormale levertestafwijkingen. Verder tonen onze gegevens dat de introductie van de thans algemeen geaccepteerde biochemische respons criteria vooralsnog niet heeft geleid tot een significante stijging van het aantal patiënten bij wie het beleid hierop werd afgestemd.

Van belang is verder erop te wijzen dat er tot op heden slechts één behandeling officieel geregistreerd is voor de ziekte PBC. Dit verklaard waarschijnlijk waarom niet-geregistreerde, maar wel potentieel effectieve medicijnen, zoals budesonide en fibraten, zelden werden voorgeschreven. Naast toenemend bewijs voor de effectiviteit van budesonide[6-8] en fibraten[26-30] lijkt ook obeticholzuur[12] een potentieel effectief geneesmiddel te zijn. De verwachting is dat in de komende jaren diverse middelen geregistreerd zullen worden voor de tweedelijnsbehandeling van PBC.

Verder bevestigde deze studie dat een adequate UDCA dosering, 13-15mg/kg/dag, van belang is.[31, 32] Ongeveer 40% van de patiënten die in eerste instantie geclassificeerd werden als non-responder voldeden na het ophogen van de dosering tot een adequaat niveau aan de criteria voor respons. Met een multivariabele analyse bevestigden we dat de UDCA dosering een belangrijke factor is voor de kans op een voldoende behandelrespons.

Ondanks dat het mogelijk is om met de biochemische respons criteria, zoals Barcelona, Paris I en II, Toronto en Rotterdam criteria,[21-25] patiënten te classificeren als responder of non-responder, kennen deze criteria ook nadelen. Zo maken ze gebruik van gedichotomiseerde variabelen, waardoor potentieel belangrijke prognostische informatie verloren gaat. Daarnaast blijken er forse verschillen te bestaan tussen de diverse criteria in welke patiënten ze classificeren als responder of non-responder.[33] In **hoofdstuk 4** wordt een nieuw ontwikkelde en gevalideerde score (GLOBE score) gepresenteerd, bestaande uit

leeftijd, serum bilirubine, alkalische fosfatase, albumine en trombocyten. Het voorspellende vermogen van deze score na 1 jaar UDCA behandeling (C statistic 0.81, 95% CI 0.79-0.83) was significant beter dan dat van de eerder genoemde respons criteria. Met de GLOBE score kunnen dus patiënten met een hoog risico op verminderde transplantatievrije overleving worden geïdentificeerd; patiënten die in potentie baat hebben bij additionele therapieën. Vanuit kostenperspectief is het tegenovergestelde ook van belang. Met de GLOBE score kunnen namelijk ook patiënten met een laag risico op een slechte uitkomst op meer betrouwbare wijze worden geïdentificeerd. De zorg van deze laag risico patiënten zou daarop afgestemd kunnen worden. Om de klinische toepasbaarheid te vergroten, kan de score makkelijk worden uitgerekend via een webapplicatie (www.globalpbc.com).

Hepatocellulair carcinoom (HCC)

De levensverwachting van patiënten met PBC wordt voornamelijk bepaald door de ontwikkeling van cirrose, portale hypertensie en HCC.[4, 34, 35] HCC staat vijfde op de lijst van de meest voorkomende vormen van kanker en staat als derde genoteerd als oorzaak voor kanker gerelateerd overlijden.[36] HCC komt vrijwel uitsluitend voor bij patiënten met een onderliggende leverziekte met ernstige fibrose of cirrose, met name geldt dit voor mannen.[37] Veel studies die gedaan zijn ten aanzien van HCC in PBC betreffen monocenter studies met relatief kleine groepen patiënten en beperken daarom de mogelijkheid tot het bepalen van betrouwbare risicofactoren.[38-48] In **hoofdstuk 5** beschrijven we de incidentie van HCC en risicofactoren voor het ontwikkelen van HCC in een grote, voor de ziekte representatieve en internationale populatie van PBC patiënten.

In overeenstemming met eerdere studies bleken het mannelijk geslacht en een ernstiger ziektestadium belangrijke risicofactoren voor het ontwikkelen van HCC. Echter, een multivariabele analyse toonde aan dat trombocytopenie en biochemische non-respons op UDCA therapie de belangrijkste risicofactoren waren. Het risico op het ontwikkelen van een HCC bleek niet alleen hoger te zijn in UDCA behandelde patiënten met non-respons, maar ook in onbehandelde patiënten die voldeden aan de criteria van non-respons. Het voldoen aan biochemische respons criteria, onafhankelijk van therapie, lijkt dus een surrogaat voor een verbeterde HCC-vrije overleving.

Biochemische respons criteria[21-25, 49] spelen dus een belangrijke rol in het voorspellen van transplantatievrije overleving en het risico op het ontwikkelen van HCC. Een belangrijke vraag ten aanzien van HCC is in hoeverre routinematige surveillance geïndiceerd geacht kan worden. Op basis van onze bevindingen adviseren we om extra beducht te zijn op HCC in 1) mannelijke PBC patiënten met biochemische non-respons op UDCA, ongeacht het ziektestadium, of mannen met cirrose, ongeacht respons en 2) vrouwelijke patiënten met ernstige fibrose of cirrose en een biochemische non-respons. Een aanbeveling voor een toekomstige studie is om te onderzoeken welke rol de GLOBE score heeft in risicostratificatie van HCC.

DE PROGNOSTISCHE BETEKENIS VAN LEEFTIJD EN GESLACHT IN PRIMAIRE BILIAIRE CIRROSE

Ongeveer 90% van de patiënten met PBC is vrouw en het merendeel is van middelbare leeftijd. Daarom is het onderzoeken van bepaalde subgroepen, zoals hele jonge, oudere, of mannelijk PBC patiënten lastig.[3, 50]

Verschillende studies hebben laten zien dat de klinische betekenis van PBC anders is voor mannen dan voor vrouwen. Vrouwen zijn vaker symptomatisch op het moment van diagnose; met name jeuk[51, 52] en vermoeidheid. Mannen daarentegen presenteren zich vaker met een verder gevorderd ziektestadium,[51-53] met een groter risico op het ontwikkelen van een HCC[54] en een verminderde transplantatievrije overleving.[50] Tevens heeft een recente studie laten zien dat het mannelijk geslacht, onafhankelijk van ziektestadium, een onafhankelijke risicofactor is voor een incomplete respons op UDCA therapie,[53] wat er op kan wijzen dat de ziekte bij mannen sneller progressief is.

Recent werd door een omvangrijke studie van het UK-PBC consortium gesuggereerd dat er een omgekeerde correlatie is tussen leeftijd en de kans op respons in vrouwen, maar niet in mannen; jongere vrouwelijke patiënten hadden een lagere kans op een biochemische respons op UDCA.[53] In **hoofdstuk 6** presenteren we een studie die ook ingaat op de vraag of leeftijd en geslacht inderdaad onafhankelijke risicofactoren zijn voor een verminderde kans op biochemische respons en transplantatievrije overleving. Wij toonden in een eveneens grote studiepopulatie aan dat gecorrigeerd voor het ziektestadium, geslacht geen onafhankelijke risicofactor is. Het lijkt er eerder op dat de lagere kans op therapierespons bij mannen verklaart wordt doordat zij zich vaker presenteren in een verder gevorderd ziektestadium; een factor waarvan eerder is aangetoond dat deze geassocieerd is met een lagere kans op therapierespons.[23, 55] Verder lieten we in deze studie zien dat jongere patiënten, ongeacht geslacht, een lagere kans op therapierespons hebben. Dit in tegenstelling tot de eerder genoemde studie van het UK-PBC Consortium, die suggereerde dat oudere vrouwen een betere respons op therapie hebben dan jongere vrouwen met PBC, echter dat dit verschil niet aanwezig is bij mannen. Dit verschil in resultaat kan mogelijk worden verklaard door het kleinere aantal mannen in de diverse patiëntengroepen die onderzocht werden in de Engelse studie.

Een reden voor een lagere behandelrespons in jongere patiënten zou kunnen zijn dat ze minder therapietrouw zijn dan oudere patiënten. Een andere verklaring zou gelegen kunnen zijn in verschillen in pathofysiologische mechanismen. Eerder werd een groep patiënten beschreven met een ernstig ductopene variant van PBC en deze patiënten bleken allen 50 jaar of jonger te zijn, relatief hoge transaminasen waarden te hebben en niet te reageren op (UDCA en corticosteroïd) therapie.[56] Een andere studie suggereerde dat ductopenie een belangrijke risicofactor is voor verminderde behandelrespons.[24] Het kan dus zijn dat

jonge patiënten met een vroeg stadium PBC vooral een “ductopeen fenotype” hebben wat in zichzelf een risicofactor is voor verminderde respons. In onze studie lieten we zien dat patiënten jonger dan 45 jaar vaak hogere serum transaminases hebben, wat een aanwijzing kan zijn voor meer inflammatoire activiteit in deze leeftijdsgroep. Dit is overigens in overeenstemming met de bevinding van Carbone et al. die vonden dat jongere patiënten vaker therapiefalen kennen dan oudere patiënten wanneer een transaminase criterium werd gebruikt.[53]

Samenvattend speelt leeftijd lijkt een belangrijke rol te spelen bij het therapeutische effect van UDCA en daarmee op de prognose op lange termijn. Het is daarom belangrijk om extra alert te zijn bij jonge patiënten met PBC. Het toevoegen van tweedelijns therapieën moet waarschijnlijk laagdrempel worden overwogen in deze patiëntengroep wanneer de standaard UDCA behandeling onvoldoende effect heeft. Additionele studies zijn nodig naar de mechanismen die ten grondslag liggen aan de verminderde behandelrespons in jongere PBC patiënten.

CONCLUSIES

Het lijkt een kwestie van tijd voordat er nieuwe geneesmiddelen worden geregistreerd als tweedelijnsbehandeling voor de ziekte PBC. Het is daarom van belang om vroeg in de behandeling UDCA- behandelde PBC patiënten te selecteren die een hoog risico hebben op het ontwikkelen van leverfalen of overlijden. Voor dit selectieproces lijken biochemische variabelen, zoals alkalische fosfatase en bilirubine, geschikt aangezien deze de capaciteit hebben om te functioneren als surrogaat eindpunten voor klinische eindpunten, zoals leverfalen of overlijden. In de kliniek kan de GLOBE score worden gebruikt om patiënten te selecteren die baat kunnen hebben bij nieuwe therapieën; een risico score bestaande uit leeftijd, bilirubine, alkalische fosfatase, albumine en trombocyten.

PBC patiënten met een goede response op UDCA behandeling hebben een relatief laag risico op het ontwikkelen van HCC. Dit geldt ook voor mannen en patiënten die zich in een gevorderd ziektestadium bevinden. Ten aanzien van de behandeling met PBC hoeven er geen verschillen te worden verwacht tussen mannen en vrouwen ten aanzien van de response op behandeling. Extra aandacht dient te worden gegeven aan jonge PBC patiënten in een vroeg ziektestadium, aangezien zij niet goed responderen op UDCA behandeling. Met name deze groep patiënten kan baat hebben bij nieuwe therapieën.

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CHAPTER 8 - APPENDICES

ACKNOWLEDGEMENTS

Ondanks dat het voorgaande in dit proefschrift, het eigenlijke proefschrift, de meeste tijd kostte om te schrijven, wordt in het algemeen aangenomen dat dit hoofdstuk, waar u zojuist naartoe gebladerd bent, als eerste en soms als enige gelezen wordt. Mocht dit laatste voor u gelden, het zij u vergeven.

Halverwege mijn promotie verhuisde jij, Harry (Prof. dr. H.L.A. Janssen), naar Toronto. Een aderlating voor onze afdeling die hierdoor een internationaal befaamd onderzoeker en hepatoloog kwijtraakte. Als promotor van diverse promovendi binnen onze afdeling bleef je gelukkig verbonden aan Rotterdam. Bij mijn onderzoek was je met name op metaniveau betrokken. Door de grote lijnen in de gaten te houden, wist je mij te motiveren binnen vier jaar mijn proefschrift af te ronden. Jouw kritische grondhouding prikkelde mij het onderste uit de kan te halen. Hartelijk dank voor je supervisie en samenwerking.

Het moet eind 2011 geweest zijn; '*Houd je van reizen?*', vroeg zij aan mij? '*Jazeker!*', sprak ik met lichte opwinding in mijn stem. '*Realiseer je je dat het werk niet altijd rozengeur en maneschijn zal zijn?*' De eerste vraag resoneerde nog na in mijn gehoorgangen en ik hoorde de verdere vragen en opmerkingen niet meer. '*Jazeker, ik houd van reizen*'.

Zo ging het niet, althans gedeeltelijk. Ik zat tegenover ene dr. B.E. Hansen. Ik kende haar nog niet, maar '*veel verstand van statistiek … be prepared*', hoorde ik om mij heen. Tijdens het gesprek kwam naar voren dat er een internationale werkgroep op het gebied van PBC moest worden opgezet (*the Global PBC Study Group*) en dat daar een promovendus voor nodig was.

Nu wordt er over statistici van alles beweerd. Zo schreef Godfried Bomans eens: "*Een statisticus waadde vol vertrouwen door een rivier die gemiddeld één meter diep was. Hij verdronk.*" Dit zou jou nu nooit overkomen Bettina. Jouw bovengemiddelde gevoel voor de exacte wetenschap bleef niet onopgemerkt, getuige de vele internationale statistische noodhulp die jij de laatste jaren verrichtte. Vele internationale werkgroepen bleken jaloers op de aangename Rotterdamse onderzoeksorganisatie met eigen statisticus en zij riepen jouw hulp in. Wij als promovendi keken met argusogen toe. Bettina, jouw hulp bij het analyseren van een grote en geavanceerde database, je adviezen in het toepassen van de juiste statistische tools, en je inzicht en het vermogen om alles uit een database te halen, hebben de basis gelegd tot enkele belangrijke artikelen op het gebied van PBC.

Het gesprek met dr. Hansen was uitermate aangenaam en vriendelijk en korte tijd later volgde een tweede gesprek met dr. H.R. van Buuren; '*een autoriteit binnen zijn vakgebied …*'. Ik had thuis voor de zekerheid nog even opgezocht wat de ziekte PBC ook alweer inhoud en ik kon met volle overtuiging zeggen: '*ja, voor zeldzame auto-*

immuunziektes van de lever, en daar is primaire biliaire cirrose er natuurlijk één van, is een internationale samenwerking uitermate verstandig'. Verschillende vraagstellingen bleken lastig te beantwoorden in kleine studiegroepen en daarom was het idee ontstaan om op internationaal niveau gegevens met elkaar te delen. Een bijkomstigheid van de internationale samenwerking zou zijn, dat ik naast vele Nederlandse centra ook naar buitenlandse centra zou moeten reizen om de patiëntengegevens op te halen en waar nodig zelf te verzamelen. Later zou blijken dat het '*…waar nodig zelf te verzamelen*' onder het kopje '*werk dat niet altijd rozengeur en maneschijn zal zijn*' viel.

Henk, in de loop van de tijd leerde ik dat jouw aanstekelijke lach na een zojuist door jezelf smakelijk vertelde anekdote (en dat waren er velen) karakteristiek te noemen is. Niemand anders dan jij, Henk, kan met gevoel voor understatement en droge humor een zaal tot lachsalvo aanzetten. Deze beide karaktereigenschappen heb ik zeer kunnen waarderen. Soms kwamen ze onbedoeld naar voren in je correspondentie. Vaak schreef jij als begeleidende email van een revisie: 'Beste Wim, zeer goed! Hierbij enkele suggesties! Groet, Henk'. Vervolgens kwam ik er bij het openen van de bijlage achter hoe rekbaar het begrip 'enkele' eigenlijk is. Bij het nadenken over nieuwe wetenschappelijke vraagstukken ten aanzien van ons internationale PBC project, was de klinische relevantie jouw drijfveer. Henk, jouw grondige en kritische beoordelingen van de manuscripten hebben mede geleid tot enkele high-impact papers.

Beste copromotoren, Henk en Bettina, met elkaar hebben we een fantastische internationale studiegroep weten op te zetten. Daarnaast hebben de vele besprekingen en etentjes uiteindelijk geresulteerd in een aantal fantastische papers en er ligt nog veel meer in het verschiet. Ik heb het zeer gewaardeerd dat tijdens de wekelijkse besprekingen die wij hadden, niet alleen wetenschappelijke zaken werden besproken en ook zeker niet alleen koffie werd gedronken. Hartelijk dank voor deze fantastische periode die ik met jullie heb mogen samenwerken.

I would like to thank all members of the Global PBC Study Group. It is an honour for me to collaborate with all of these worldwide experts in the field of liver research. Thank you very much for your warm welcome and hospitality during my center visits and stays. I hope to continue being part of this unique study group. Angela (dr. A.C. Cheung), thank you for working together on a collaborative paper.

In particular, I would like to thank Palak (dr. P.J. Trivedi). Dear friend, it was a great pleasure for me to collaborate with you. Our highly effective and smooth way of collaboration resulted in a fantastic publication in Gut. Thanks a lot for this outstanding piece of teamwork. I admire your enthusiasm, unpretentiousness and integrity. These unique characteristics and your dedication and talent for research are a successful combination. I wish you all the best together with your wife and son. I am looking forward to collaborate with you in the near future!

Uiteraard wil ik alle artsen danken die participeerden in de Nederlandse multicentrische PBC studiegroep. De Nederlandse PBC database, in de afgelopen tientallen jaren zorgvuldig opgebouwd, was de basis voor diverse proefschriften en is ook onderdeel van dit proefschrift.

Graag wil ik prof.dr. H.J. Metselaar, prof.dr. U.H.W. Beuers en prof.dr. E.W. Steyerberg danken voor het plaatsnemen in de kleine commissie. Prof.dr. J.L.C.M. van Saase, prof. F. Nevens en dr. G.M. Hirschfield wil ik danken voor het zitting nemen in de promotiecommissie. Dear Gideon (dr. G.M. Hirschfield) and prof. F. Nevens, I am honoured that both of you are willing to take place in the PhD committee for my thesis defence.

Er zijn nog diverse andere mensen die ik wil bedanken voor hun hulp tijdens mijn promotieonderzoek. De studenten Lotte Kehrer, Marjolijn Leeman, Bibi Bouwen en Esther Zoutendijk hielpen mij met het verzamelen en invoeren van patiëntengegevens in een database, waarvoor hartelijk dank! Irene en Edith, dank voor jullie ondersteuning vanuit het Clinical Trial Bureau bij diverse klinische studies die we hebben verricht op het gebied van PBC en PSC. Melek, Heleen en Esther, dank voor jullie hulp tijdens de Hepatitis B, PSC en PBC trialpoli's die ik mocht doen tijdens mijn promotie. De samenwerking heb ik als prettig ervaren. Marion en Margriet, jullie als secretaresses bleken onmisbaar. Dank voor jullie gezelligheid en fantastische inzet tijdens diverse onderdelen van mijn promotie.

Beste Rob (dr. R.A. de Man), dank voor het gestelde vertrouwen mij te willen opleiden tot maag-, darm- en leverarts. In mei 2015 ben ik begonnen met de vooropleiding interne geneeskunde in het Sint Franciscus Gasthuis te Rotterdam onder supervisie van dr. A.P. Rietveld. Graag wil ik hem danken voor het prettige opleidingsklimaat in het SFG.

Dat de tweeërlei betekenis van *promoveren* ook daadwerkelijk van toepassing bleek op het arts-onderzoeker zijn, werd direct duidelijk na mijn aanstelling. De eerste betekenis *het verwerven van de academische graad van doctor* was iets wat in de loop van de tijd wel gebeuren zou. De tweede betekenis bleek onverwachts in werking te treden toen ik qua werkplek *promoveerde* naar de dakpoli. Die benaming slaat op de ooit als noodvoorziening gebouwde keet bovenop het polikliniekcomplex. De dakpoli had in de tussentijd een behoorlijk aantal illustere voorgangers gehuisvest en gezien de populariteit van de dakpoli mocht het als voorrecht worden beschouwd daar een plek toebedeeld te krijgen. Ik kreeg een plek op kamer Ca-413. Dat ik de in de tussentijd gedateerde computer 'mocht' overnemen, was slechts bijzaak. De bijbehorende opstarttijd van circa 15 minuten nam ik voor lief. In de tussentijd probeerde ik wat van de intelligentie op te snuiven die hier toch zeker nog moest rondwaaien. Dat dit regelmatig tot niesbuien leidde, werd niet in de minste plaats veroorzaakt door het stof in, op en tussen de ruimte-innemende ordners met onderzoeksgegevens van weleer, soms decades geleden. Deze tot trofeeën gepromoveerde ordners, die de planken

boven mijn werkplek volledig in bezit namen, bleken museumstukken geworden te zijn die onder geen beding verplaatst mochten worden, laat staan vernietigd.

In de jaren die volgden op de dakpoli, deelde ik de kamer achtereenvolgens met Dewkoemar, Vivian, Annemarie, Ingrid en Anniek. Met elkaar doorleefden we praktisch elk jaargetijde tot in z'n volle wasdom; de gure wind tijdens stormachtige herfstdagen die door kieren en gaten waaide die ons tot dat moment niet waren op gevallen, de vrieskou in de winter die de werking van de radiatoren haast volledig neutraliseerde en de verzengende hitte tijdens de zomermaanden die een constante stroom zweetdruppels op onze voorhoofden produceerde. Misschien was het voorjaar wel de periode waarin we de minste extreme ervoeren en onze grootste productiviteit behaalden. Zeer gewaardeerde kamergenoten van weleer, het was mij een waar genoegen met jullie een kamer te hebben mogen delen. Dank voor de vele gezellige en motiverende gesprekken.

Daarnaast dank ik al mijn collega arts-onderzoekers. Aan de ene kant was er een strikte scheiding tussen de MD-ers en de hepa's, gezien de afzonderlijke casusbesprekingen en congressen. Aan de andere kant waren daar de vele gezamenlijke activiteiten. Een onvolledige lijst zou kunnen zijn: dagelijkse lunches waar uiteenlopende zaken besproken werden, jaarlijkse picknick in het Erasmuspark, sportactiviteiten waar meedoen belangrijker was dan winnen, skivakanties waar skiën slechts één van de programmaonderdelen was, sinterklaasavonden inclusief het declameren van persoonlijke en ontroerende gedichten, kerstmaaltijden zonder kalkoen en de borrels na werktijd bij Rotterdamse, befaamde cafés. Collega's, jullie gaven kleur aan de soms wat eenzame werkzaamheden van mij als promovendus. Allen hartelijk dank voor de fantastische tijd op 'het dak'.

Dan de Hepa's: Heng (3 jaar lang mijn 'roomie' tijdens de vele congressen), Lisanne, Michelle, Raoel en Willem-Pieter, met elkaar hebben we vele congressen bezocht. Postertjes, praatjes, borrels en steden verkennen. In eerste instantie werden we op sleeptouw genomen door de oude rotten in het vak: Ad, Daphne, Edith, Ludi, Milan, Pauline en Roeland en later namen wij Maren, Margo en Marjolein mee op stap (dat de nieuwelingen allemaal vrouw bleken te zijn met de beginletters 'Mar-', moest puur toeval zijn). Dank voor de bijzonder leuke, gezellige en leerzame periode waarin hoogte- en dieptepunten met elkaar werden gedeeld!

Nu is het jouw beurt, Maren. Je startte in september 2014 en gedurende het afgelopen jaar hebben we samen kunnen werken aan diverse projecten. Het is voor mij een hele geruststelling te weten dat jij mij opvolgt. Je enthousiasme en talent zullen er zeker voor zorgen dat the Global PBC Study Group succesvol zal blijven. Ik zie uit naar het vervolg van onze samenwerking!

Graag wil ik mijn vrienden danken voor hun medeleven en interesse in mijn onderzoek. Dit doe ik, met een enkele uitzondering daar gelaten, zonder specifieke namen te noemen (jullie weten wie ik bedoel)! Alex, dank voor je ontwerp van het *the Global PBC Study Group* logo.

Je hebt waarschijnlijk nooit kunnen bevroeden dat één van jouw creaties internationaal zou gaan en momenteel gebruikt wordt op menig officieel document. Willem, samen beleefden we menig Afrikaans en Chinees avontuur. Graag wil ik niet onbenoemd laten dat jij, als pragmatisch ingesteld medicus, degene bent met wie ik de eerste stappen in de wetenschappelijke wereld zette. Momenteel woon en werk je in Congo samen met Joanne en jullie zoon Lukas. Ik heb veel bewondering en respect voor jullie gedrevenheid en passie om onder soms erbarmelijke omstandigheden primaire medische zorg te verlenen. Het ga jullie goed.

Dan mijn beide paranimfen. Wouter, ongekend dat we als vrienden en oud-huisgenoten al zolang dezelfde weg bewandelen. We startten in 2004 tegelijkertijd met de opleiding geneeskunde en na onze opleiding hebben we allebei een jaar als ANIOS interne geneeskunde gewerkt (jij in het SFG en ik in het Ikazia ziekenhuis) alvorens afzonderlijk van elkaar een promotieplek bij de MDL te bemachtigen (jij bij de MD en ik bij de L). Als klap op de vuurpijl werden we in 2014 allebei aangenomen voor de opleiding tot maag-, darm- en leverarts (jij in Leiden en ik in Rotterdam). Het is voor mij dan ook bijzonder dat je vandaag mijn paranimf wilt zijn! Succes met het afronden van je eigen proefschrift.

Mark, sinds jaar en dag zijn we vrienden en hebben we veel gemeenschappelijke interesses. Echter, de medische wereld is voor jou, als civiel ingenieur, een ver-van-je-bed-show. Dat je dit graag zo houdt, blijkt wel als ik zo nu en dan een medische casus in geuren en kleuren aan je vertel. Ik waardeer je interesse en betrokkenheid bij mijn onderzoek. Fijn dat je vandaag naast me wilt staan als paranimf!

Lieve pa & ma, Lydia & Lennart & Jort, Roel en Annemarij, met elkaar hebben we veel mooie en verdrietige momenten gedeeld. Ik waardeer onze hechte onderlinge band en ik ben enorm blij dat jullie vandaag getuigen zijn van mijn promotie. Daarnaast wil ik jullie danken voor jullie onophoudelijke interesse tijdens de afgelopen jaren.

Tevens dank ik mijn opa & oma Vastenhoud en mijn schoonouders, Niek & Miranda en opa en oma Rook, voor hun oprechte interesse voor deze lastige materie. Het is voor mij een eer dat jullie allen aanwezig kunnen zijn bij de verdediging van mijn proefschrift.

Lieve Caroline, er zijn maar weinig niet-medici die zoveel weten over PBC als jij. De afgelopen jaren waren je begrip en geduld eindeloos groot. Het gebeurde toch niet zelden dat ik weer naar het buitenland moest voor een ziekenhuisbezoek of congres, of dat ik 's avonds en in de weekenden aan mijn onderzoek werkte. Jouw interesse in mijn onderzoek was een belangrijke motivatiebron om het succesvol af te ronden. Woorden zijn te beperkt om uit te drukken wie jij voor mij bent. Ik zie uit naar onze toekomst, samen met onze zoon Lucas.

CHAPTER 8 - APPENDICES

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9. **Lammers WJ**, Cheung AC, van Buuren HR, Trivedi PJ, LaRusso N, Ponsioen CY, Floreani A, Hirschfield GM, Corpechot C, Mayo MJ, Invernizzi P, Battezzati PM, Parés A, Nevens F, Thorburn D, Mason AL, Lindor KD, Lleo, Poupon R, Janssen HLA, Hansen BE. Age, but not sex, alters response to UDCA in patients with primary biliary cirrhosis – an international and multicenter study. *In progress.*
10. Van Buuren HR, **Lammers WJ**, Harms MH, Hansen BE. Surrogate end points for optimal therapeutic response to UDCA in primary biliary cirrhosis. *Accepted for publication.*

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CURRICULUM VITAE

Wim Lammers werd geboren op 3 juli 1986 te Ede. Nadat hij zijn VWO diploma behaalde in 2004 aan het Wartburg College te Rotterdam, startte hij met de studie geneeskunde aan de Erasmus Universiteit Rotterdam. Tijdens zijn studie liep hij stage op diverse afdelingen van Hôpital de Gahini in Gahini, Rwanda. In 2008 verrichtte hij in het kader van het doctoraal examen zijn afstudeeronderzoek op de afdeling Traumatologie van het Ruijin Hospital in Shanghai, China. Na het doorlopen van zijn coschappen legde hij in 2010 de Hippocratische eed af. Na zijn studie zette hij zijn eerste schreden als basisarts op de afdeling Interne Geneeskunde van het Ikazia Ziekenhuis te Rotterdam. Een jaar later startte hij een promotieonderzoek met als onderwerp *risk stratification in primary biliary cirrhosis* op de afdeling maag-, darm- en leverziekten van het Erasmus MC te Rotterdam onder supervisie van prof. dr. H.L.A. Janssen, dr. H.R. van Buuren en dr. B.E. Hansen. Sinds mei 2015 is hij in opleiding tot maag-, darm- en leverarts (opleider: dr. R.A. de Man). De tweejarige vooropleiding volgt hij in het Sint Franciscus Gasthuis te Rotterdam (opleider: dr. A.P. Rietveld). Wim is getrouwd met Caroline en Lucas is hun zoon.

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PHD PORTFOLIO

Name: Willem Johannes Lammers
Affiliation: Erasmus University Medical Center, Rotterdam
Department: Gastroenterology and Hepatology

Promotor: Prof.dr. H.L.A. Janssen
Copromotors: dr. H.R. van Buuren and dr. B.E. Hansen
PhD period: 2012-2015

1. PhD training	Year	Workload
General academic skills		
Research Integrity and Good Clinical Practice (GCP) Basiscursus Regelgeving en Organisatie van Klinisch onderzoek (BROK cursus) *Erasmus MC, Rotterdam*	2012	24 hours
Biomedical English writing and communication course *Erasmus MC, Rotterdam*	2013	40 hours
Research skills		
Onderwijs in methodologie van klinisch onderzoek *Working group Clinical Research 2.0, Erasmus MC, Rotterdam*	2013-2014	20 hours
Biostatistics for clinicians *Netherlands Institute for Health Sciences (NIHES), Erasmus University Rotterdam*	2012	40 hours
Survival analysis for clinicians *Netherlands Institute for Health Sciences (NIHES), Erasmus University Rotterdam*	2012	40 hours
Oral presentations		
Alkaline phosphatase values are a surrogate marker in prediction of transplant-free survival in patients with primary biliary cirrhosis – an international, collaborative analysis *48th Annual Meeting of the European Association for the Study of the Liver (EASL), Amsterdam, the Netherlands*	2013	36 hours
Improving prognosis prediction in primary biliary cirrhosis (PBC) – the Global PBC Study Group *Avondsymposium Cholestatische Leverziekten, Amsterdam, the Netherlands*	2013	24 hours
Defining optimal laboratory response criteria in UDCA-treated primary biliary cirrhosis. Results of an international multicenter long-term follow-up study *Twice annual meeting of the Netherlands Association of Hepatology (NVH), Veldhoven, the Netherlands*	2013	12 hours

1. PhD training	Year	Workload
Alkaline phosphatase values are a surrogate marker in prediction of transplant-free survival in patients with primary biliary cirrhosis – an international, collaborative analysis *Twice annual meeting of the Netherlands Association of Hepatology (NVH), Veldhoven, the Netherlands*	2013	12 hours
Validation of alkaline phosphatase and bilirubin values as a surrogate endpoint in primary biliary cirrhosis - an international, collaborative study *64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Washington, DC, United States of America*	2013	36 hours
Defining optimal laboratory response criteria in UDCA-treated primary biliary cirrhosis. Results of an international multicenter long-term follow-up study *64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Washington, DC, United States of America*	2013	36 hours
Sub-stratification of hepatocellular carcinoma risk in men with primary biliary cirrhosis: results of an international multicenter study *Twice annual meeting of the Netherlands Association of Hepatology (NVH), Veldhoven, the Netherlands*	2014	12 hours
New model to identify UDCA-treated primary biliary cirrhosis patients in need of additional therapy. Results of an international follow-up study of 4119 patients *Twice annual meeting of the Netherlands Association of Hepatology (NVH), Veldhoven, the Netherlands*	2014	12 hours
New model to identify UDCA-treated primary biliary cirrhosis patients in need of additional therapy. Results of an international follow-up study of 4119 patients *65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America*	2014	36 hours
New risk score to determine PBC patients with non-response to ursodeoxycholic acid treatment - the Global PBC Study Group *Avondsymposium cholestatische leverziekten, Vienna, Austria*	2015	24 hours
Poster presentations		
Alkaline phosphatase values are a surrogate marker in prediction of transplant-free survival in patients with primary biliary cirrhosis – an international, collaborative analysis *48th Annual Meeting of the European Association for the Study of the Liver (EASL), Amsterdam, the Netherlands*	2013	32 hours
Impact in daily practice of the concept of treatment response in primary biliary cirrhosis. A national cohort study *49th Annual Meeting of the European Association for the Study of the Liver (EASL), London, United Kingdom*	2014	32 hours
Evaluation of the concept of biochemical response in UDCA treated patients with primary biliary cirrhosis. A Dutch multicenter study *Monothematic Conference on Primary Biliary Cirrhosis of the European Association* *for the Study of the Liver (EASL), Milan, Italy*	2014	32 hours

1. PhD training	Year	Workload
Sub-stratification of hepatocellular carcinoma risk in men with primary biliary cirrhosis: results of an international multicenter study *65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD),* *Boston, MA, United States of America*	2014	32 hours
Identification of PBC patients in need of additional therapy during the course of UDCA treatment – an international, multicenter study *50th Annual Meeting of the European Association for the Study of the Liver (EASL), Vienna, Austria*	2015	32 hours
Risk factors for hepatic decompensation in patients with PBC. Results of an international follow-up study of 2326 patients *50th Annual Meeting of the European Association for the Study of the Liver (EASL), Vienna, Austria*	2015	24 hours
The impact of age and sex on biochemical response and transplant-free survival in patients with PBC: results from the Global PBC Study Group *50th Annual Meeting of the European Association for the Study of the Liver (EASL), Vienna, Austria*	2015	24 hours
Elevation of alkaline phosphatase during follow-up is an early predictor of hyperbilirubinaemia and of clinical endpoints in primary biliary cirrhosis – an international study *66th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, United States of America*	2015	24 hours
Attended (inter)national conferences		
47th Annual Meeting of the European Association for the Study of the Liver (EASL). Barcelona, Spain	2012	28 hours
The Liver Meeting 2012, 63th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America	2012	28 hours
48th Annual Meeting of the European Association for the Study of the Liver (EASL). Amsterdam, the Netherlands	2013	28 hours
Twice annual meeting of the Netherlands Association of Hepatology. Veldhoven, the Netherlands	2013	12 hours
The Liver Meeting 2013, 64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Washington, DC, United States of America	2013	28 hours
49th Annual Meeting of the European Association for the Study of the Liver (EASL). London, United Kingdom	2014	28 hours
Monothematic Conference on Primary Biliary Cirrhosis (PBC) of the European Association for the Study of the Liver (EASL). Milan, Italy	2014	12 hours
Twice annual meeting of the Netherlands Association of Hepatology. Veldhoven, the Netherlands	2014	12 hours
The Liver Meeting 2014, 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America	2014	28 hours
50th Annual Meeting of the European Association for the Study of the Liver (EASL). Vienna, Austria	2015	28 hours

1. PhD training	Year	Workload
The Liver Meeting 2015, 66th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). San Francisco, CA, United States of America	2015	28 hours
Awards		
Registration bursary awarded for the best abstracts by young investigators during the 47th Annual Meeting of the European Association for the Study of the Liver (EASL)	2012	
Registration bursary awarded for the best abstracts by young investigators during the 48th Annual Meeting of the European Association for the Study of the Liver (EASL)	2013	
Full bursary awarded for the best abstracts by young investigators during the 49th Annual Meeting of the European Association for the Study of the Liver (EASL)	2014	
Full bursary awarded for the best abstracts by young investigators during the monothematic conference on primary biliary cirrhosis of the European Association for the Study of the Liver (EASL)	2014	
Young investigator travel award during the 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD)	2014	
Full bursary awarded for the best abstracts by young investigators during the 50th Annual Meeting of the European Association for the Study of the Liver (EASL)	2015	
Attended seminars and workshops		
Avondsymposium Cholestatische Leverziekten. Barcelona, Spain	2012	4 hours
27th Erasmus Liver Day. Rotterdam, the Netherlands	2012	6 hours
10th Post-AASLD symposium. Rotterdam, the Netherlands	2012	2 hours
Avondsymposium Cholestatische Leverziekten. Amsterdam, the Netherlands	2013	4 hours
28th Erasmus Liver Day. Rotterdam, the Netherlands	2013	6 hours
11th Post-AASLD symposium. Rotterdam, the Netherlands	2013	2 hours
5e Lagerhuisdebat Hepatitis B en C. Utrecht, the Netherlands	2013	3 hours
1st 'PBC: past, present and future' meeting. London, United Kingdom	2014	6 hours
Avondsymposium Cholestatische Leverziekten. London, United Kingdom	2014	4 hours
1st Post-EASL symposium. Amsterdam, the Netherlands	2014	2 hours
6e Lagerhuisdebat Hepatitis B en C. Utrecht, the Netherlands	2014	3 hours
2nd 'PBC: past, present and future' meeting. Vienna, Austria	2015	6 hours
Avondsymposium Cholestatische Leverziekten. Vienna, Austria	2015	4 hours
Dinner Pensant, regionale onderwijsbijeenkomsten. Rotterdam, the Netherlands	2012-2015	28 hours
Memberships		
Dutch Society of Gastroenterology (NVGE)		
Dutch Society of Hepatology (NVH)		
The European Association for the Study of the Liver (EASL)		
The American Association for the Study of Liver Diseases (AASLD)		

2. Teaching activities	Year	Workload
Lecturing		
International multi-center cohort studies *PhD day, department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam*	2012	6 hours
Surrogate endpoints in primary biliary cirrhosis *Early morning session, department of Gastroenterology and Hepatology, Azienda Ospedaliera di Padova, Padua, Italy*	2013	6 hours
First analysis. Database and syntax *PhD day, department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam*	2013	6 hours
Multi-center studies on location *PhD day, department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam*	2014	6 hours
Pathophysiology of cholestasis *Minor 3rd year curriculum, study of Medicine, Erasmus University Rotterdam. Rotterdam, the Netherlands*	2014	18 hours
State of the art lecture: primary biliary cirrhosis, current insights into diagnostics and treatment *Nascholingsavond regionale MDL opleiding, Nijmegen, the Netherlands*	2015	18 hours
Supervising graduation project		
Marjolijn Leeman, medical student, Erasmus University Rotterdam Master's thesis, 2013-2014 about *How the concept of biochemical response influenced the management of primary biliary cirrhosis in 831 patients over three decades*		60 hours

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List of Abbreviations

AASLD	American Association for the Study of Liver Disease
ALT	Alanine aminotransferase
AMA	Antimitochondrial autoantibody
ANA	Antinuclear autoantibody
APRI	AST to platelet ratio index
AST	Aspartate transaminase
CI	Confidence interval
FXR	Farnesoid-X receptor
γ-GT	Gamma-glutamyl transpeptidase
HBV	Hepatitis-B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis-C virus
HR	Hazard ratio
ICD	Individual centre dataset
IQR	Interquartile range
LLN	Lower limit of normal
MELD	Model of end stage liver disease
NL	Natural logarithm
NRI	Net reclassification improvement
OCA	Obeticholic acid (INT-747)
PBC	Primary biliary cirrhosis
PDC-E2	Pyruvate dehydrogenase complex – E2 subunit
PPAR	Peroxisome proliferator-activated receptors
PT	Prothrombin time
UDCA	Ursodeoxycholic acid
ULN	Upper limit of normal