Prognosis and Treatment of Primary Biliary Cholangitis

a new name, a new era

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Colophon

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van Primaire Biliaire Cholangitis
een nieuwe naam – een nieuw tijdperk

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CHAPTER 1
GENERAL INTRODUCTION

Based on:
Improving Prognosis in Primary Biliary Cholangitis – therapeutic options and a treatment strategy

Risk stratification and Prognostication in Primary Biliary Cholangitis

Surrogate Endpoints for Optimal Therapeutic Response to UDCA in Primary Biliary Cholangitis

Primary biliary cholangitis (PBC), formerly known as primary biliary cirrhosis, was first described by Addison & Gull in 1851[1]. PBC is a chronic liver disease that is characterized by a non-suppurative destructive cholangitis, and its autoimmune features. The disease can lead to severe ductopenia, accompanied by progressing fibrosis, ultimately leading to cirrhosis, liver failure, and death.

DIAGNOSIS

A clinician should consider PBC in case of persistent, unexplained cholestatic abnormalities - in particular alkaline phosphatase - in serum liver tests. The diagnosis of PBC can be finalized when anti-mitochondrial antibodies (AMA) at a titre of >1:40 are also present in serum. Up to 95% of patients with PBC have detectable anti-mitochondrial autoantibodies against pyruvate dehydrogenase complex E2 in serum, making this a hallmark in the diagnosis[2]. Given the high specificity of the combination of these serological markers for PBC, there is no need for a liver biopsy when both are present[3]. In case of AMA-negativity together with unexplained increased serum alkaline phosphatase, or in case of suspicion of an autoimmune overlap syndrome, a liver biopsy can be helpful. Histologically, PBC is characterized by chronic, non-suppurative inflammation, destroyed interlobular and septal bile ducts, and florid duct lesions. The latter can often be identified at early stages of the disease. When there is more progression of disease, fibrosis and bile duct loss can be found[4, 5].

NOMENCLATURE

Until recently, primary biliary cholangitis was known as primary biliary cirrhosis. The latter name was first proposed in 1950, as most patients at that time presented with advanced liver disease[6]. However, over the years, this name became an anachronism, as the majority of patients nowadays are diagnosed in an early stage of disease, in which cirrhosis is not present[7-9]. Many patients struggled with the stigmatization that came with the denomination cirrhosis, as in society it is often associated with excessive use of alcohol. These arguments regarding the misnomer of cirrhosis have led to an extensive re-evaluation of the suitability of the former disease name. “Chronic non-suppurative destructive cholangitis” was often proposed, but although this name was highly accurate, most professionals and patients agreed that it would be desirable to stick to the well-known abbreviation PBC. Eventually, consensus was found in “primary biliary cholangitis”, despite the pleonasm that is secluded in this new name[10].

EPIDEMIOLOGY

PBC is the most common of the autoimmune diseases of the liver. A systematic review of epidemiological studies on PBC across North America, Europe, Australia, and Asia showed an estimated incidence of 0.9-5.8 per 100,000 people per year[11, 12]. The prevalence is estimated at 2-58 patients per 1000 people, but in many countries, a trend of rising incidence and prevalence is observed. There are, however, large geographical differences in both incidence and prevalence, for which an explanation is lacking to date. The disease predominantly affects women, with a reported male to female ratio of 1:10, although recent studies show a trend over time towards a higher percentage of males[13]. Patients are typically diagnosed in their fifties, but the disease can affect patients as young as twenty, as well as elderly.

AETIOLOGY AND PATHOPHYSIOLOGY

PBC is considered to be an autoimmune disease, in light of its strong association with other autoimmune diseases such as CREST, Sjögren's disease and rheumatoid arthritis[14], the strong female preponderance, and the presence of anti-mitochondrial antibodies in approximately 95% of patients[15]. Studies suggest an etiological cohesion between genetic predisposition[15, 16] and environmental factors, given the geographical clustering of PBC and associations between PBC and exposure to hair dyes, nail polish and cigarette smoking, as well as infectious agents such as Escherichia coli, Mycobacterium gordonae, and retroviruses[17-19]. However, these associations were neither strong, nor was a causative relation ever confirmed. The pathogenesis seems to evolve through interacting immunological and biliary pathways, leading to cell injury and chronic cholestasis[20]. Although much is still unknown, some of these pathways have been unraveled and have led to several pharmacological targets that are being discussed here. Firstly, it is well-established that patients with PBC encounter immunological intolerance to biliary epithelial cells, which relates to the small duct cholangitis, progressive bile duct destruction and cholestasis[21]. Loss of tolerance to mitochondrial antigens, most often the pyruvate dehydrogenase complex-E2, is characteristic of PBC and is reflected by elevated serum levels of anti-mitochondrial antibodies in ~95% of patients. The reason for this loss of tolerance, however, is yet unclarified.

Secondly, the 'biliary umbrella' theory has been an important step in better understanding of the biliary pathways in this disease[22]. It explains that under physiological conditions, an intact exchange of Cl- and HCO3- and an intact biliary glycocalyx – together forming a biliary 'umbrella'- are vital to prevent invasion of the toxic hydrophobic bile acid monomers that are present in human bile. In PBC, this bicarbonate umbrella is malfunctioning. Reduced expression of the anion exchanger 2 (AE2), which is responsible for Cl-/HCO3- exchange, on biliary epithelial cells is observed, leading to less bicarbonate excretion and subsequently

a more toxic composition of the bile. Cholangiocytes are consequently exposed to higher concentrations of toxic bile salts, which endangers the structure of cell membranes and mitochondria, and thereby stimulates apoptosis of the cholangiocytes. In turn, AE2 expression in biliary epithelial cells can be further suppressed by hydrophobic bile acids through inducement of biliary epithelial cell senescence, which subsequently leads to production of different interleukins and thereby to bile duct inflammation.

Another step in identifying pharmacological targets has been made through further comprehension of the mechanisms involved in the gut-liver axis. More specifically, the discovery that nuclear hormone receptors directly regulate genes that are involved in the homeostasis of bile acids made an impact[23]. The farnesoid X receptor (FXR) is a nuclear receptor which acts as a key transcriptional sensor of bile homeostasis, and is predominantly expressed in the liver and small intestine. Chenodeoxycholic acid and cholic acid are endogenous ligands for FXR in humans. Through suppression of CYP7A1 and upregulation of FGF19, FXR ligation inhibits bile acid uptake, bile acid synthesis, hepatic inflammation, and development of tissue fibrosis, and upregulates pathways associated with bile acid export, hepatic regeneration, and tumour suppression[24]. Alterations in nuclear receptor signaling may contribute to the pathogenesis of PBC. Although evidence for dysfunction of the aforementioned nuclear receptors in PBC is not established, activation was found be protective in animal models mimicking PBC. Other nuclear receptors found to be important in the regulation of bile acid metabolism include the peroxisome proliferator-activated receptor (PPAR), that regulates pathways inhibiting both inflammation and bile acid synthesis and enhancing phospholipids secretion, and the pregnane X receptor (PXR), mainly involved in detoxifying pathways and inhibition of biliary secretion.

Both the results of sibling studies in PBC, and the fact that first-degree relatives of PBC patients carry an increased risk of developing the same disease, indicate that genetic factors contribute to the development of the disease[25]. Genome-wide association studies (GWAS) and smaller genetic studies have identified several genetic risk loci for PBC, but more specific studies are needed for further implementation of these findings[26-28]. Personalized genomics will likely identify more nuclear receptor polymorphisms that link the pathogenesis of PBC to an altered bile metabolism, and might uncover other therapeutic targets.

SYMPTOMS

The initial clinical presentation of patients with PBC ranges from incidentally detected abnormal liver chemistry on routine testing to a first presentation of end-stage liver disease complications such as variceal bleeding. However, cirrhosis-unrelated symptoms also represent an important clinical problem for patients with PBC. The most common symptoms are pruritus and fatigue, but a range of other symptoms can occur including arthralgia, dry eyes and mouth (''sicca complex''), nonspecific abdominal pain, and unsightly xanthelasma. It has been well established that also cognitive symptoms, sleep disturbance, and social isolation may affect patients' quality of life[29]. As debilitating PBC-related symptoms largely dictate the burden of disease, improving patient's quality of life by ameliorating these symptoms is one of the key goals of ongoing studies. Aforementioned limitations in the understanding of the pathogenesis of the disease is reflected by the current limited therapeutic options to relieve symptoms satisfactorily in a large subset of symptomatic patients.

NATURAL HISTORY

Timely diagnosis of PBC can be challenging as patients may remain asymptomatic for many years[30], all the while the disease may silently progress[31]. Histologically, chronic destructive non-suppurative cholangitis is typical for PBC[4], but liver biopsy no longer has a place in the standard diagnostic work-up[20, 32]. In contrast with the situation several decades ago, the majority of patients are nowadays diagnosed in an early stage of disease[33, 34]. When left untreated, patients with PBC are likely to develop cirrhosis, potentially resulting in cirrhosis-related complications, liver failure and death. In the follow up of one of the first clinical trials, liver biopsies were performed on a regular basis. This study showed that 31% of the patients with histological stage 1 and 50% of the patients with stage 2 progressed toward cirrhosis within 4 years[35]. Few studies have assessed the incidence of cirrhosis-associated complications in untreated patients with PBC. A Chinese study, in which 26 patients were included that were not or inconsistently treated, showed that the median time until decompensation was approximately 5 years. Unsurprisingly, decompensating events are associated with poor subsequent survival. The median survival after such events was reported to be approximately two years, which is comparable with the natural history of other liver diseases[36, 37]. An English long-term observational study of 770 untreated patients with PBC reported a median survival after PBC diagnosis of 9.3 years, and a standardized mortality ratio of 2.9 (95% confidence interval 2.6-3.2)[38]. In multivariate Cox regression analysis, several factors were reported to be predictive of subsequent patient survival. This model included age at diagnosis, albumin, bilirubin, and alkaline phosphatase. Earlier studies have reported that survival of patients who are symptomatic at time of diagnosis is much shorter than of those who present asymptomatic, with a median survival of 7.5 and

16 years respectively[30], but a larger cohort described by *Mahl et al*. found that although the absence of symptoms at time of diagnosis represented an earlier stage of disease, it was not associated with better prognosis[39]. Other long-term follow up studies also showed that initially asymptomatic patients are likely to eventually develop pruritus and fatigue and that their survival is impaired as compared to the general population[40]. In 1989, *Dickson et al*. developed the Mayo Risk Score, a model including the clinical parameters bilirubin, albumin, prothrombin time, age, and severity of oedema, which predicted short-term survival in untreated PBC based on the data of 312 patients of which 125 died during a median follow-up of 4.1 years[41]. The outcome of this continuous score allows estimation of survival up to seven years. Patients stratified into low, medium and high risk groups by this model had median 5-year survival rates of approximately 90%, 60% and 10% respectively. The Mayo Risk Score has long been considered very instrumental in predicting prognosis in PBC and has in fact been frequently used in the evaluation of treatment efficacy by comparing the Mayo-predicted survival to the actual observed survival with treatment.

THERAPIES

In the mid 1970's, little was known about the pathophysiology of PBC and copper deposition was thought to play an important pathophysiological role. Hence, the first randomized controlled trial (RCT) in PBC studied the effectivity of the copper-chelating agent D-penicillamine[42]. Treatment benefit was not confirmed in large multicenter studies, and the drug came with serious adverse effects[43,44]. Since then, the understanding of PBC has improved considerably, but today the etiology of the disease still remains largely unknown. Consequently, specific etiology-based curative therapies are currently not available and the search for new and better therapeutic options is ongoing. A number of non-specific therapies, however, have shown significant potential to modify the course of the disease and have substantially changed perspectives for patients with PBC. Although ultimate therapeutic benefit is measured by a reduction of the risk of mortality or liver transplantation (LT) in randomized controlled trials (RCTs), earlier measures of outcome in PBC can include biochemical and histological parameters, non-invasive markers of liver fibrosis, and incidence of relevant clinical events.

First-line treatment

Beneficial effects of treatment with bear bile were already recognized in ancient China. In the previous century, following the identification of ursodeoxycholic acid (UDCA) as the primary bile acid in bear bile and its biochemical structure and properties, UDCA was extensively used for treating (dissolution) of cholesterol bile stones. Early anecdotal reports of Japanese researchers on treatment effects of UDCA on liver biochemistry in the sixties and seventies remained virtually unnoticed. The first reports of Ulrich and Maria Leuschner, showing

beneficial effects of UDCA on laboratory parameters, published in 1985, marked the real beginning of the UDCA era in PBC[45]. Today, UDCA is the most extensively studied therapeutic agent in PBC. It is a choleretic and hydrophilic endogenous bile acid that has multiple sites and mechanisms of action. Firstly, it stimulates secretion of bile acids from hepatocytes, preventing hepatocyte injury, apoptosis, and necrosis and subsequent inflammation and fibrosis. Secondly, through the activation of AE2 transporters, treatment results in UDCA enrichment and expansion of the bile acid pool and thereby induces a less toxic bile composition. Subsequently, the bile is less harmful to its environment of cholangiocytes, ameliorating the degree of cholangiocellular injury, inflammation, and proliferation. Thirdly, immunomodulatory effects have been observed, possibly influenced by UDCA-induced activation of the glucocorticoid receptor[21]. Based on the extensive experience obtained with this drug over the past decades, 13-15 mg per kilogram of UDCA daily is currently recommended as the standard treatment for PBC by international guidelines[20, 32]. A multi-center RCT with 2 years of follow up (n=146) reported a 57% reduction of ALP in UDCA-treated patients, as opposed to a 5% increase in the placebo arm. Bilirubin decreased slightly with 9% in UDCA-treated patients, while there was a marked worsening of 68% in the placebo-arm (both $p<0.001$)[46]. Numerous other placebo-controlled trials also showed that UDCA induced significant reductions of bilirubin, ALP and transaminases. Although regression of histological stage has not been reported, most studies observed significant differences in portal inflammation, bile duct paucity and piecemeal necrosis in favor of UDCA-treated patients[47-50]. Although there were some conflicting results[48-50], several studies concluded that UDCA delays progression of fibrosis and histological stage[47, 51]. In a cohort study of over 4000 patients, no difference in cumulative HCC incidence between UDCA-treated and untreated patients ($p=0.972$) was established. However, it should be noted that HCC is a rare event in PBC with an overall incidence rate of 3.4 per 1000 patient years[52]. Despite these overall promising results, however, RCTs failed to show a therapeutic benefit on transplant-free survival, as did most meta-analyses[46, 50, 51, 53-60]. In one combined analysis (without predefined inclusion criteria) of three specific RCTs, *Poupon et al.* reported a reduced risk of LT or death (RR 1.9, $p<0.001$) in patients treated with UDCA, but only in case of advanced disease[61]. However, as PBC is a slowly progressing disease, the follow-up of most of the trials has not been adequate to reliably evaluate treatment effect on survival, especially in patients with an early stage of disease who nowadays represent the majority of patients. As the feasibility of new studies that could adequately assess treatment effect on LT and death is hugely complicated by the low prevalence and the slowly progressive nature of the disease, a quest for valid and accurate surrogate markers for clinical outcomes was inevitable in PBC. It has been long established that bilirubin is an important prognostic marker for clinical outcome in PBC[62, 63]. However, elevation of bilirubin is often not observed in early stages of disease, and is thereby a relatively late marker of disease progression. In 2006, *Pares et al.* reported that 61% of patients of an observational cohort of 192 UDCA-treated patients showed either normalization or a decrease of at least 40% of their serum

ALP after one year of treatment. The study demonstrated that achievement of this newly proposed response criteria was associated with a better LT-free survival compared to the survival of so-called 'non-responders' to UDCA (RR 5.5, p=0.004)[64]. In the following years, several other criteria for biochemical response to UDCA were constructed, based on observational studies of UDCA-treated patients. These studies all reported that achievement of biochemical response (according to their specified criteria after one or two years of treatment) was significantly associated with an improved LT-free survival as compared to patients with inadequate response, as well as compared to their expected survival without UDCA based on the Mayo Risk Score, thereby suggesting a therapeutic benefit of UDCA[65-69].

Being an endogenous substance, UDCA is very well tolerated and real intolerance is a rare event. Severe adverse effects are not known. An occasional patient may experience some abdominal discomfort, flatulence, or diarrhea upon initiation of treatment, but these symptoms are usually transient. In case of persistent discomfort, switching to a different brand of UDCA or lowering the dosage might be considered. There is no evidence or other ground to assume that the effect of apportioning the total dosage of 13-15 mg/kg UDCA as a multiple dosage regimen is better than a single dosage once daily[70].

Second-line treatments

For the majority of patients, treatment with UDCA is effective and the life expectancy of patients with a complete biochemical response is comparable to that of sex and age matched counterparts in the general population[71]. However, *Trivedi et al.* reported in a recent cohort of mainly UDCA-treated patients that approximately 40% of patients had developed cirrhosis after 10 years, with the subsequent risk of cirrhosis-associated complications[8]. Approximately one third of patients has an inadequate biochemical response to treatment with UDCA[71]. For these patients, there is a need for additional treatment to reduce the risk of premature mortality and LT. Several therapies have recently been proposed as second-line therapy, most often in addition to treatment with UDCA. Since most of these therapies are relatively new, long-term data is scarce. Therefore, most of the evidence for these treatments available today is based on the assumption of surrogacy of ALP and bilirubin for long-term outcome.

Budesonide

Budesonide is a highly potent glucocorticoid that has a 90% first-pass effect through the liver in healthy individuals, with a receptor binding activity 15-20 times greater than prednisolone. Because only 10% of the substance reaches the systemic circulation, the potential risk of systemic side effects is much lower than in classical steroids[72]. This is of importance, especially giving the intrinsically higher risk of bone density problems in women with PBC[73]. Notably, it has been demonstrated in vitro that budesonide and UDCA are synergistic in upregulating AE2 expression. Budesonide was the first potential second-line therapeutic drug in PBC to

show promising, but also conflicting results in trials. In 1999, the first placebo-controlled trial of 39 patients assessed budesonide as add-on therapy to UDCA at 9 mg/day in patients with early PBC, and reported significant reductions of ALP and histological improvements in the treatment arm. The UDCA monotherapy group (n=19) had a 30% reduction of ALP after two years, as opposed to 50% in the budesonide add-on arm (n=20)[74]. However, in a subsequent open label study (n=22) of patients with a persistently elevated ALP despite UDCA treatment, only a marginal improvement of ALP was reported after one year of add-on budesonide (9 mg/day), and this was accompanied by worsening of osteoporosis, and not by improvement of bilirubin or prognosis as reflected by the Mayo Risk Score[75]. *Rautiainen et al*. later reported encouraging results of statistically significant improvement of fibrosis (25%) and histological stage (22%) in a 3-year non-blinded controlled study in non-cirrhotic patients with PBC (n=77) However, this finding might have been influenced by a markedly high rate of progression in the UDCA monotherapy arm (20% histological stage deterioration and 70% increased fibrosis). There was no significant difference regarding the change in grade of inflammation and ALP between UDCA monotherapy the intervention arm, and bone density was not assessed[76]. A 3-year phase III double-blind RCT was terminated early because of slow recruitment and as a result, insufficient power to detect a significant histological difference between treatment groups, although normalization occurred in 35% of the treated arm[77]. Side effects of budesonide mostly include steroid-related effects such as bruises, acne, thinning of skin and weight gain. Importantly, the pharmacokinetics of budesonide are different in patients with advanced disease. Aside from potential bone density issues, serious adverse advents such as portal vein thrombosis have been described after administration of budesonide to cirrhotic patients with PBC[78]. Therefore, treatment with budesonide is regarded contraindicated in late stage disease.

Fibrates

Fibrates are carboxylic acids and are primarily known for their ability to reduce serum lipid levels. In 1993, fibrates were first suggested as a potential therapy for patients with cholestatic liver disease after an improvement in ALP was observed in patients that were treated for hyperlipidaemia[79]. Later, fibrates were shown to act as ligand for the nuclear receptor PPAR. PPAR is known to exist in three isoforms (alpha (α), beta (β)/gamma (γ), and delta (δ)). These isoforms are encoded by distinct genes and have different patterns of distribution. The available types of fibrates have different specificities for the PPAR isoforms, and thereby induce different effects.

Fenofibrate is a selective PPARα-agonist. PPARα is involved in several pathways influencing lipid metabolism, pathways regulating synthesis and detoxification of bile acids, and pathways regulating inflammatory responses. Over the past 15 years, several pilot studies have assessed the effect of 80-200mg fenofibrate treatment in PBC as add-on to UDCA in patients with an incomplete biochemical response to UDCA alone[80-85]. All reported an improvement

of up to 50% in ALP, but these studies were of small sample size and mostly with short follow up. Two more recent observational studies also reported results regarding the effect of fibrates on the (estimated) prognosis[86, 87]. Although *Cheung et al.* described a favourable adjusted decompensation-free survival (HR 0.15, p=0.03) among those treated with add-on fenofibrate (n=46) as opposed to those treated with UDCA alone (n=74), it should be noted that the number of clinical events in this study was limited and ongoing biochemical deterioration was observed in both study arms. *Hegade et al.* (n=23) found no significant improvement in estimated LT-free survival based on the UK-PBC score (a continuous score estimating transplant-free survival, based on biochemistry), despite a significant decrease in ALP after one, two and three years of follow-up[86]. A placebo-controlled phase-III trial is currently ongoing in China.

Bezafibrate is a non-selective PPAR-agonist, targeting the three isoforms in equivalent molar concentrations. Although many pilot studies, mainly from Japan, have suggested beneficial effects on biochemistry associated with bezafibrate as add-on treatment to UDCA[88-95], the strongest evidence in favor of efficacy of any add-on fibrate treatment originates from the recently presented results of BEZURSO. This 1:1 placebo-controlled trial (n=100) assessed the add-on effect of 400mg/day bezafibrate to UDCA in patients with an incomplete response to UDCA, which was defined as ALP >1.5x the upper limit of normal (ULN), or AST >1.5x ULN, or bilirubin>ULN, after one year of UDCA (*Paris II response criteria*[58]). In total, 67% of bezafibrate-treated patients achieved normalization of ALP, and 30% even showed a complete biochemical normalization of bilirubin, ALP, aminotransferases, albumin and other parameters after two years. Treated patients also showed improvement of liver stiffness measurements (p<0.01). Notably, there was a remarkable decrease in pruritus in the treatment arm as opposed to no change in the placebo group[96]. Beneficial effects of bezafibrate were also observed in a prospectively followed Spanish cohort with 48 patients over a median period of 38 months. Important observations were a major effect on pruritus and absence of a clear favorable response in more advanced disease[93].

Other fibrates that are currently being studied include MBX-8025 (seladelpar, a selective PPARδ-agonist) and GFT-505 (elafibranor, a dual PPARα/δ-agonist). Unlike PPARα that is predominantly expressed in hepatocytes, PPARδ is also expressed in cholangiocytes, Kupffer, and stellate cells. Consequently, it may regulate additional pathways involved in bile acid absorption and secretion, function of cholangiocytes and may induce anti-fibrotic and anti-inflammatory effects on Kupffer and stellate cells. A recent 12-week phase II RCT in which seladelpar was dosed in patients with an incomplete response to UDCA (n=41) showed that both 50mg and 200mg daily induced >50% reduction in ALP. This study was terminated, however, after occurrence of significant transaminase increases in patients treated with the active drug[97]. None of the other aforementioned studies found evidence for serious adverse events related to fibrate treatment. Flares of both transaminases and creatinine

have been reported, but were transient after discontinuation of treatment. The flares in creatinine imply that the use of fibrates should be avoided in case of renal impairment. One study reported an accelerated increase in bilirubin in cirrhotic fenofibrate-treated patients, suggesting that caution with the use of fenofibrates might be warranted in these patients[87]. In clinical practice, the choice for a specific fibrate is often limited by the fact that subtypes are not uniformly available in most countries.

Farnesoid X receptor agonists

FXRs have a key role in the regulation of the synthesis, secretion, detoxification and transportation of bile acids. Chenodeoxycholic acid is the most potent endogenous bile acid for FXR. Obeticholic acid (OCA) is a synthetic derivative of chenodeoxycholic acid and is >100-fold more potent, and thus a strong FXR-agonist. Besides activating FXR, OCA also induces expression of fibroblast growth factor (FGF)-19, possibly explaining the anti-inflammatory effects that have been observed in murine models. OCA was granted FDA approval in 2016, based on the results of an international multi-centre phase III RCT of 216 patients. In this study, OCA was assessed as add-on treatment to UDCA in patients with persistent abnormalities in their liver biochemistry under treatment with UDCA monotherapy[98]. The approval of OCA represented a major breakthrough in the treatment of PBC, with OCA being the first FDA-approved drug for PBC since the introduction of UDCA nearly three decades earlier. Subjects in the 5mg and 10mg treatment arms showed significant improvement of ALP (-113U/L and –130U/L vs. -14U/L in the placebo arm, $p<0.001$) and total bilirubin (-0.3µmol/L and -0.5µmol/L vs. +2.0µmol/L in the placebo arm, $p<0.001$). As mentioned, survival benefit of add-on OCA has yet to be confirmed, for which a long-term follow-up is currently ongoing. Importantly, OCAs most common side effect in patients with PBC is pruritus, a symptom that already is prevalent in PBC and can be debilitating. However, when the dosage was titrated up to a maximum of 10 mg/day, treatment discontinuation due to pruritus was rare. Another potentially worrisome effect of OCA includes alteration of the lipid metabolism, resulting in a significant decrease of high-density lipoprotein cholesterol and an increase of low-density lipoprotein, of which long-term implications are unclear[99]. Recently, the Food and Drug Administration (FDA) has warned that dosing of OCA should be altered in patients with moderate to severe decreases in liver function. In case of Child-Pugh B or C, patients should be started on 5 mg once weekly, rather than daily as advised in other PBC patients.

Other strategies

Immunosuppressive and immunomodulatory agents

PBC is considered to be an autoimmune disease[100]. Logically, the effects of several immunosuppressive and immunomodulatory agents besides budesonide have been assessed over the past decades. Immunosuppressive and immunomodulatory drugs that were evaluated in RCTs include azathioprine[93], methotrexate[101], thalidomide[102], colchicine[103],

corticosteroids[104-106], cyclosporine[107], malotilate[108], and mycophenolate mofetil[109], and were mostly studied as add-on treatment to UDCA. Unfortunately, the results of these studies have been largely disappointing, with a lack of improvement of patients' biochemistry, histology, and overall survival, and/or reporting unacceptable risk of adverse events. These trials have resulted in the recommendation not to use any of the aforementioned agents as standard therapy for PBC. However, low dose prednisone might be considered in case of (features of) an autoimmune overlap syndrome[20]. Considering the assumption that PBC is primarily an autoimmune mediated disease, these overall disappointing results are remarkable and may suggest that autoimmune features only partially reflect the true nature of the disease.

Biologicals and other experimental studies

In other fields such as inflammatory bowel disease, the use of biologic agents that target cytokines and other pathways of immune responses, have caused a major breakthrough in treatment. In PBC, the possibilities and potential of biological therapies are currently being studied extensively, with promising results in preclinical studies. Several studies are currently assessing safety and clinical effect in humans, but patience is likely required for potential implementation into clinical practice. Antiretroviral drugs have also been evaluated for the treatment of PBC, but results have been conflicting[110].

Liver transplantation

When pharmacological interventions fail to adequately delay disease progression, PBC can eventually lead to end-stage liver disease and liver failure, at which point LT is the only therapeutic intervention that can prevent death. Refractory pruritus and hepatocellular carcinoma are other much more rare indications for LT in PBC. In the first decades following the first human LT in 1963, primary biliary cholangitis was the leading indication for LT in Europe, accounting for 30-50% of all LTs[111]. The gradual introduction of UDCA as the standard of care is thought to have made a substantial impact. Nevertheless, a minority of patients with PBC does still require LT to prevent premature mortality today. Also, a recent study showed that waitlist mortality is higher in PBC as compared to most other etiologies except chronic hepatitis C and alcoholic liver disease[112]. Graft and patient survival after LT for PBC are generally good. The European Liver Transplantation Registry reported a 1, 5 and 10 year patient survival of 86%, 80%, and 71% respectively, and results reported by the United Network for Organ Sharing in the United States were highly comparable[112, 113]. Disease recurrence after LT occurs in 11-42% of all transplanted patients[114].

RISK STRATIFICATION

In the setting of risk stratification the aim is to estimate the likelihood of a clinical event taking place. Assessment of the risks and risk parameters allow identification of patients or patient groups with mild or a more progressive disease pathway, and thereby allow the targeting of care. Below the association and impact of various biochemical and clinical factors with clinical events is reviewed. *Shapiro et al.* (1979) were the first to start a long history of studying factors associated with disease progression in PBC, recognizing the association between serum bilirubin levels and survival (**Table 1**)[62]. They found that patients with bilirubin levels >2 mg/dl in two subsequent measurements within 6 months had an average survival of 4.1 years, whereas the average survival was 2.1 and 1.4 years when two subsequent measurements were above 6 or 10 mg/dl, respectively. Furthermore, they showed that the behavioural pattern of bilirubin is characterized by two distinct phases: a phase in which bilirubin remains stable for many years followed by an 'acceleration' phase with rapidly increasing values cumulating in death within a few years[62]. Similar patterns are observed in other end-stage liver diseases[115]. Confirming these phases, *Harms et al.* (2016) showed (n=3529) that the curve breaking point of bilirubin was found at a bilirubin 1.6 times the upper limit of normal (ULN). From this breaking point onward there were a median of 19 months before a clinical endpoint occurred[116]. This suggests that bilirubin is a "late" biomarker, i.e. increasing only shortly before a clinical event, and thereby less applicable for early detection of progression of disease.

Alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) are considered to be early markers of disease[20]. In meta-analyses of 4845 patients *Lammers et al.* showed that both alkaline phosphatase (>2.0 x ULN) and bilirubin (> 1.0xULN) are independent predictors of liver transplantation and death[34]. Although outcomes were best predicted by biochemistry measured one year after initiation of UDCA, ALP and bilirubin measured at other time points remained strongly associated with clinical outcomes. Importantly, ALP levels held additive prognostic value to bilirubin and this effect was independent of sex, follow-up time, presenting age, UDCA treatment and disease stage. Thus, this landmark paper showed that ALP and bilirubin levels are strongly associated with long-term outcomes in PBC. Both are considered the most robustly validated markers of disease activity (ALP and bilirubin) and disease stage (bilirubin) in PBC. ALP and bilirubin are accepted to be 'reasonably likely to predict clinical benefit' in PBC and are used as an endpoint in clinical trials[117].

The concept of biochemical response

Angulo et al. were the first to recognize that changes in biochemical parameters during UDCA treatment were associated with clinical outcome [118].In a cohort of 180 UDCA-treated patients, they showed that patients with serum ALP >2 times the upper limit of normal

Table 1. Prognostic factors in primary biliary cholangitis

Prognostic factor	Prognostic significance	Strengths✓ and limitations✖
Demographics		
Male sex vs. female sex[52-54]	Associated with UDCA non-response (72% vs. 80%, p<0.005)[52] (Attributable to more advanced disease)[53] Increased HCC risk in male non-responders and male cirrhotic patients[54]	✖Lack of external validation
Younger age at diagnosis[52, 53]	Response rate in patients < 50 years old < 68% vs. 86% in aged > 60	✖Lack of external validation
Symptomatic patients[55-60]	Fatigue: may be associated with worse transplant-free survival[55] Pruritus: conflicting data[56-60].	✖Lack of standardization in symptom definitions
Serological markers		
Anti-gp210[61, 62]	Hepatic decompensation subtype (associated with 6-fold increase in LTx/death)	✖Unclear whether prognostic impact is independent to UDCA response; needs replication in large-scale cohort
Anti-centromere[61, 63]	Portal hypertension subtype	
Biochemical variables		
Bilirubin[37]	Surrogate marker of disease activity and stage	✓Externally validated and considered the most robust markers of disease activity in PBC ✖May not be a realistic markers for the risk stratification of early-stage populations
Alkaline phosphatase[37]	Surrogate marker of disease activity	
Albumin [31, 34, 37]	Low levels are independent predictor of liver-related mortality	
AST and ALT [6, 7, 51]	Elevated levels (after 12 months UDCA) associated with worse survival/liver-related events	
APRI[36]	>0.54 associated with death/LTX (HR 2.75)	✓Externally validated ✓Prognostic value additive to biochemical response
Markers of disease stage		
ELF	Each point increase associated with 3-fold increase of adverse events[64]	✖Unclear whether marker of disease activity or stage ✖Calibration not assessed ✖Unclear cost-effectiveness compared to LFTs and TE
Liver stiffness measurement [65, 66]	LSM progression associated with prognosis[65] kPa > 9.6 associated with decompensation, LTx or death (HR 5.1; 95% CI 1.5-15.9) [65] Significantly improves newly introduced risk stratification models[66]	✖Large inter-operator variability ✖Poor discrimination in subtle changes in liver fibrosis ✖Overestimates of fibrosis stage in cholestasis[67]
Histological parameters[6, 9, 50, 68-70]	Advanced histological stages: associated with poor prognosis[50, 68] Interface hepatitis: associated with the development of cirrhosis and liver transplantation or liver-related death and[34, 70] Premature ductopenic variant associated with progressive disease[69] Baseline ductopenia (>50% loss) predicts histological progression[9]	✖Biopsy is an invasive and costly procedure ✓ Prognostic value additive prognostic to prognostic models ✖Lack of external validation

HCC, hepatocellular carcinoma; LTx, liver transplantation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio; HR, hazard ratio; ELF, enhanced liver fibrosis; LFTs, liver function tests; TE, transient elastography; LSM, liver stiffness measurement; kPa, kilopascal.

(ULN) after six months of therapy were more likely to encounter severe disease progression (11% vs. 33%, p<0.04). In 2006, a Spanish study found (n=192) that a 40% reduction of ALP after one year of treatment was associated with a similar survival as that of matched controls from a general population (Barcelona criteria). In contrast, the prognosis of those who did not meet these criteria was worse than that of a general population (relative risk for liver transplantation or death 7.47 (95% CI 1.87-29.78)). Since then, several response criteria have been proposed, all with different combinations of biochemical variables to capture incomplete response to UDCA and thereby identifying patients that are at risk of events[64-68, 119, 120] (**Table 2**). Most criteria evaluate biochemical response after one year of UDCA. However, the optimal time point for biochemical evaluation has yet to be determined and it may already be possible to assess response to therapy after 6 months[121]. *Leuschner et al.* showed that approximately 80% of decrease of alkaline under UDCA treatment occurs within 6 months of UDCA therapy, suggesting that most criteria are best applied after at least 6 months of therapy[74]. The Paris-I criteria are the most accurate and thoroughly validated dichotomous criteria and are considered superior in discriminating patients into low- and high-risk categories for events[66, 122-124]. However, the optimal response criteria may differ between patients and study populations. For example, Paris II criteria were designed for early stage disease patients[68]. Combined analyses of various proposed criteria showed they have independent prognostic significance, suggesting that none of these criteria is optimal measure of response[125]. Furthermore, some criteria are mainly focused at the assessment of response to treatment and do not incorporate markers of disease severity or stage (e.g. albumin and/or bilirubin). These criteria may not sufficiently capture the baseline difference in survival that is associated with difference stages of disease[126]. Nonetheless, biochemical response criteria provide a readily available way to identify patients that are likely to benefit from additional therapies or clinical trials.

Serum markers of fibrosis

Serum markers of fibrosis provide an outcome on a continuous outcome scale, potentially providing more information than the categorized histological disease stages. Although several markers, including serum hyaluronate, the ELF (enhaced liver fibrosis) score, and AST/ALT ratio have been studied[127-131], the most promising serum marker of fibrosis is the aspartate aminotransferase-to platelet ratio (APRI). An APRI of >0.54, as a surrogate for liver fibrosis and portal hypertension, is an important non-invasive marker and prognostic factor associated with cirrhotic complications, death and liver transplantation in PBC patients[122, 132]. APRI is associated with outcome independent of response to treatment with UDCA and thus imparts additional prognostic value to existing biochemical response criteria[122]. An APRI of >0.54 after one year of UDCA an therapy is associated with an almost 3-fold increase in risk of death or liver transplantation.

Table 2. Proposed criteria for the assessment of biochemical response to UDCA in PBC

Criteria	Formula	Time point (months UDCA)	Clinical endpoint / Non-response, %	PBC group / n	Follow-up time	Remarks (strengths✓ and limitations✖)
Rochester/ Mayo, 1999[3]	ALP <2.0 × ULN	6	Liver transplant-free survival / Not reported	All / 180	Range 0.4-7.0 years	✖Short FU time ✖Small sample size ✖Does not incorporate disease severity
Barcelona, 2006[4]	>40% decrease of ALP or normalization	12	Liver transplant-free survival / 39	All /192	Range 1.5-14 years	✓Matched survival with general population ✖Does not incorporate disease severity ✖ 17 endpoints (9 died, 8 fulfilled criteria for liver transplantation (treatment failure))
Paris I, 2008[6]	ALP <3.0 × ULN and AST <2.0 × ULN and normalization of bilirubin	12	Liver transplant-free survival / 39	Advanced / 292	Median 5.3 (range 1.0-21.5) years	✓Externally validated [5, 7, 36, 51, 73] ✓Combination of multiple variables ✓Systematic determination of cut-offs ✖Limited to advanced disease stage
Rotterdam, 2009[5]	Normalization of abnormal bilirubin and/or albumin	12	Liver transplant-free survival / 24	All / 375	Median 9.7 (range 1.0-17.3) years	✓Long-term FU, prospective large sample ✓Incorporation of disease stage ✓Included patients from general- (39) and university (7) hospitals
Ehime, 2009/2011[8, 10]	≥70% decrease of γ-GT	6	Liver transplant-free survival / 52[8] and 79[10]	Asymptomatic / 83 [8] and 134[10]	Mean 5.2±4.4 years[8] and median 4.6 (range 0.8-24.3) years	✓Systematic determination of cut-off ✖Small sample size [8] ✖Short FU time ✖Does not incorporate disease severity
Toronto, 2010 [9]	ALP ≤ 1.67 × ULN	24	Histological progression and liver transplant-free Survival / 43	All / 69	Mean 9.4 years	✓Systematic determination of cut-off and optimal timing of assessment (1 or 2 years of therapy) ✓Paired liver biopsy ✖Small sample size ✖Does not incorporate disease severity
Paris II, 2011 [7]	ALP and AST ≤ 1.5 × and normalization of bilirubin	12	Liver transplant-free survival,ascites, variceal bleeding,encephalopathy, HCC / 52	Early PBC (Ludwig I and II) / 165	Median 7 (range 1.6-20.3) years	✓Applicable in early disease stage presenting patients ✓Systematic determination of cut-off ✓Inclusion of multiple endpoints significant to prognostication in PBC ✖Only 11 endpoints
Momah/ Lindor, 2012 [11]	ALP ≤ 1.67 × and bilirubin ≤ 1mg/dL	12	Development of varices, ascites, encephalopathy, liver transplantation or death /48	All / 73	Mean of 3 years	✓Use of multiple endpoints significant to prognostication in PBC ✓Systematic determination of cut-off ✖Small sample size

UDCA, ursodeoxycholic acid; ALP, alkaline phosphatase; ULN, upper limit of normal; AST, aspartate aminotransferase; γ-GT, gamma-glutamy transpeptidase; HCC, hepatocellular carcinoma.

Liver stiffness measurement

Liver stiffness measurement (LSM) with vibration-controlled transient elastography provides a simple measure of liver fibrosis stage, especially in severe fibrosis and cirrhosis[130, 133, 134]. In an Italian cohort study (n=120), LSM by transient elastography was better in identifying any grade of fibrosis and cirrhosis (AUROC 0.89, 0.92 and 0.99 for fibrosis stage II, III and IV, respectively) than non-invasive surrogate markers of fibrosis such as APRI (AUROC 0.66,0.67 and 0.84 for fibrosis stage II, III and IV, respectively) and the AST/ALT ratio (AUROC 0.53,0.57 and 0.58 for fibrosis stage II, III and IV, respectively). Subsequently, *Corpechot et al.* showed LSM values above 9.6 kPA carry a hazard of 5 for adverse outcomes (decompensation, liver transplantation or death)[133]. In addition, progression of liver stiffness at a cut-off of 2.1 kPa/year is associated with an increased risk of adverse outcomes[133]. Recent studies suggest that poor biochemical response is associated with higher rates of LSM progression and that LSM progression is able to predict clinical outcomes in PBC independently of UDCA response[133, 135]. Preliminary data suggests that LSM significantly improves risk stratification of newly established prognostic scores[136]. However, transient elastography is not uniformly available in all clinics, requires experience and may be unreliable in obese patients. Moreover, cholestasis can falsely increase LSM values resulting in inaccurate estimates of fibrosis severity[137]. These factors currently limit the possibilities of including LSM into prognostic tools for the general clinician.

Continuous models predicting transplant-free survival

Early risk prediction models were mostly developed for end-stage PBC, primarily focus on short-term survival, and do not incorporate biochemical response or disease activity (e.g. ALP). Therefore, in this era with mostly UDCA-treated and early-disease stage presenting patients, these models may not be sufficient. Recently, two new models were proposed that overcome these shortcomings. In 2015, the GLOBE score was introduced (www.globalpbc.com). This model was constructed using a derivation cohort of 2488- and a validation cohort of 1634 UDCA-treated patients, and comprises age, bilirubin, albumin, alkaline phosphatase, and platelet count after 1 year of UDCA treatment as independent predictors of liver transplantation or death in UDCA-treated patients[123]. Also introduced in 2015, the UK-PBC risk score (www.uk-pbc.com) was developed in a nation-wide cohort of 1916 patients (derivation cohort) and validated in a cohort of 1249 UDCA-treated PBC patients, this score predicts the risk of liver-related death or liver transplantation with a model comprising baseline albumin and platelet count, as well as bilirubin, transaminases, and alkaline phosphatase after 1 year of UDCA therapy[131]. With C-statistics of >0.8, both these models have superior predictive performances for incomplete response to UDCA compared to previously proposed dichotomous criteria[123, 131]. The scores use variables on a continuous scale resulting in more conservation of predictive information. The outcomes of the scores have a continuous scale too and thus provide gradual individualized estimates of survival, rather than crude differentiation into high- and low-risk groups. Importantly,

they take into account biochemical response to UDCA by incorporating biochemistry after one year of therapy, thus combining predictive information of both disease severity and response to treatment. Therefore, these models are better able to accurately predict survival than previously introduced models and biochemical response criteria. The GLOBE score was initially constructed to estimate the risk of death or liver transplantation after 1 year of UDCA therapy. However, recent analyses indicate that the score can also be used to risk stratify UDCA-treated patients at later points in time[138-140]. An advantage of the GLOBE score is its use of age-specific thresholds beyond which survival significantly deviates from a sex and age-matched general population. The score presents the median survival of this matched population at 3, 5, 10, and 15 years.

SCOPE AND AIMS OF THIS THESIS

This thesis focuses on the natural history, risk stratification, and treatment of patients with primary biliary cholangitis. The aims of this thesis were to assess:

1. the predictive value of bilirubin within the normal range on transplant-free survival among patients with PBC
2. the incidence of and risk factors for cirrhosis-related complications among ursodeoxycholic-acid treated patients with PBC
3. the association between ursodeoxycholic acid and transplant-free survival in patients with PBC, both in relative and absolute measures
4. biochemical response and clinical outcome of PBC patients treated with fenofibrate and bezafibrate
5. the estimated survival benefit induced by treatment with obeticholic acid in patients with PBC
6. time trends in liver transplantation for patients with PBC.

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CHAPTER 2
BILIRUBIN WITHIN THE NORMAL RANGE IS PREDICTIVE OF SURVIVAL IN PRIMARY BILIARY CHOLANGITIS

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ABSTRACT

Objective In primary biliary cholangitis (PBC), bilirubin and alkaline phosphatase (ALP) are widely established as independent predictors of prognosis. Current treatment goals do not aim for normalization of surrogate markers, as their association with survival has not been defined.

Methods Patients from the GLOBAL PBC Study Group cohort were utilized, comprising of long-term follow-up data from European and North American centers. Ursodeoxycholic acid (UDCA)-treated and untreated patients with bilirubin levels ≤1×ULN at baseline or 1 year were included. The association of normal ALP with transplant-free survival was assessed in a sub-group with ALP≤1.67×ULN at 1 year. Optimal thresholds of bilirubin and ALP to predict liver transplantation (LT) or death were evaluated.

Results There were 2281 patients included in the time zero cohort and 2555 patients in the 1-year cohort. The bilirubin threshold with the highest ability to predict LT or death at 1 year was 0.6×ULN (HR 2.12, 95% CI 1.69-2.66, P<.001). The 10-year survival rates of patients with bilirubin ≤0.6×ULN and >0.6×ULN were 91.3% and 79.2%, respectively (P<.001). The risk for LT or death was stable below bilirubin levels of 0.6×ULN yet increased beyond this threshold. UDCA-induced reduction in bilirubin below this threshold was associated with an 11% improvement in 10-year survival. Further, ALP normalization was optimal, with 10-year survival rates of 93.2% in patients with ALP≤1×ULN and 86.1% in those with ALP 1.0-1.67×ULN.

Conclusion Attaining bilirubin levels ≤0.6×ULN or normal ALP are associated with the lowest risk for LT or death in patients with PBC. This has important implications for treatment targets.

INTRODUCTION

Primary biliary cholangitis (PBC) is an autoimmune cholestatic liver disease that is characterized by chronic non-suppurative inflammation of the small intrahepatic bile ducts[1]. The disease usually has a slow progressive course, which may eventually lead to cirrhosis and ultimately liver failure or premature death in the absence of liver transplantation (LT). However, the prolonged number of years it may take for patients to develop such clinical outcomes poses a significant obstacle in randomized controlled trials that aim to evaluate the clinical benefit of therapeutic interventions. Due to these feasibility concerns, various surrogate markers have been evaluated for their prognostic value on clinical outcomes[2]. Such surrogate markers can allow the risk stratification of patients without the need for an extended follow-up period and can be implemented by health care providers or in clinical trials to promptly assess the need and benefit of a therapeutic agent.

It is widely established that bilirubin is an independent predictor of prognosis in both ursodeoxycholic acid (UDCA)-treated and untreated patients with PBC[2–4].The normalization of bilirubin prompted by UDCA has been associated with improved transplant-free survival[4]. Furthermore, bilirubin has been established as a surrogate endpoint that is "reasonably likely to predict clinical benefit" and the threshold that best predicted liver transplant-free survival was reported to be the upper limit of normal (ULN)[2]. Normal bilirubin is also a component of multiple response criteria, such as the Rotterdam, Paris-I, and Paris-II criteria[5–7].

Abnormal bilirubin levels are observed during later stages of PBC and are indicative of impaired liver function[8]. Over the past decades, however, there has been an increase in the proportion of patients that present with normal bilirubin levels over the years and this group now represents the majority of patients with PBC[9].Since bilirubin is usually not elevated above the ULN until later stages of the disease, it is considered to be an inadequate marker for risk stratification in early stage PBC. The prognostic value of bilirubin below the ULN has not been previously assessed. Thus, the aim of this study was to evaluate whether bilirubin levels within the normal range (≤1×ULN) are associated with survival in patients with PBC.

MATERIALS AND METHODS

Population and study design

This is a retrospective study on the predictive value of normal bilirubin for survival in patients with PBC. We utilized the Global PBC Study Group database, which includes long-term follow-up data of PBC patients from 16 centers across Europe and North America. To evaluate the association between normal bilirubin and survival, we included UDCA-treated and untreated patients diagnosed with PBC according to internationally accepted guidelines and whose bilirubin levels were normal (≤1×ULN as defined by each local center) at time zero or 1 year after study entry [8,10,11]. Those with short follow-up (<6 months), short-term treatment with UDCA (discontinued), absent laboratory values, unknown dates of important clinical events, overt overlapping features of autoimmune hepatitis (AIH), or other concomitant liver diseases were excluded from the study. Patients were allocated to two independent cohorts based on the time point(s) at which their bilirubin levels were normal (time zero and 1 year). The inclusion of patients into each cohort is not mutually exclusive, as patients may have had normal bilirubin levels at both time points. This study was conducted in accordance with the 1975 Declaration of Helsinki. The protocol was approved by the institutional research board at all participating centers as per local regulations.

Data collection

In the Global PBC Study Group database, time zero (study entry) is defined as the date UDCA was initiated in treated patients and the date of the first visit in untreated patients. At study entry, the following data were available: sex, age at diagnosis, anti-mitochondrial (AMA) antibody serological status, liver histology, biochemical disease stage (according to Rotterdam criteria[5]), and UDCA therapy. The following laboratory parameters were collected every 6-12 months: total bilirubin, alkaline phosphatase (ALP), albumin, aspartate aminotransferase (ALT), alanine aminotransferase (AST), and platelet count. Histological data obtained from liver biopsies conducted within 12 months of study entry were staged according to *Ludwig et al.* and *Scheuer's* criteria[12,13]. The completeness and accuracy of the data was established by visits to participating centers.

Statistical analyses

The primary endpoint was a composite of LT and all-cause mortality. Survival was defined as an absence in LT and all-cause mortality. Patients without an event at the end of follow-up and those who were lost to follow-up were censored at their last visit. The predictive value of normal bilirubin on the primary endpoint was initially analyzed based on the bilirubin quartiles corresponding to each cohort. The survival rates across quartiles were estimated with a Kaplan-Meier curve and compared with a log-rank test. Multivariable Cox proportional hazards' regression (hazard ratio [HR] with 95% CI) analyses were performed to

adjust for potential confounding variables: age at study entry, sex, year of diagnosis, UDCA therapy, ALP, and geographical region.

To test the hypothesis of a threshold and to determine the optimal threshold for bilirubin within the normal range two approaches were followed: 1) bilirubin levels at baseline and 1 year of follow-up were dichotomized according to various thresholds ranging from 0.3 to 0.9 ×ULN in 0.01 increments. Multivariable Cox proportional hazards' regression analyses were employed to estimate the risk for LT or death associated with each threshold. The C-statistic was calculated to evaluate the performance of each threshold in predicting survival and the threshold with the best performance was determined by the highest C-statistic. 2) To assess bilirubin on a continuous spectrum and test the hypothesis that the predetermined bilirubin threshold is the point at which the risk for LT or death increases, bilirubin was inserted into the Cox regression as a restricted cubic spline function with four knots. This analysis included patients with bilirubin levels above the ULN to illustrate how their risk for a poor prognosis differs relative to those with bilirubin below the ULN. The restricted spline function was repeated with crude bilirubin levels (mg/dL).

All analyses were adjusted for age at study entry, sex, year of diagnosis, UDCA therapy, ALP, and geographical region. Laboratory data included in the multivariable model that were not normally distributed were log transformed. Sensitivity analyses of the predetermined bilirubin threshold by multivariable Cox regression were performed in additional sub-groups stratified by the ULN of bilirubin (<1.2mg/dL and ≥1.2mg/dL [75th percentile of ULN of bilirubin across centers]), age at study entry (≤55 years and >55 years), sex, treatment (UDCA-treated and UDCA-untreated), histological stage (I-II and III-IV), and ALP (≤1.67×ULN and >1.67×ULN). Furthermore, sensitivity analyses were performed for bilirubin at 2-5 years after the start of follow-up.

For illustrative purposes, Kaplan-Meier analyses were conducted to describe the survival rates associated with bilirubin levels at baseline and 1 year (normal bilirubin [≤/> the threshold] and abnormal bilirubin). Patients with abnormal bilirubin were included for reference purposes. The distribution of the clinical events (LT, liver-related death, or liver-unrelated death) at 10 years within each bilirubin group was also evaluated.

An additional analysis was conducted in UDCA-treated patients whose baseline bilirubin levels were above the predetermined threshold and stratified based on their bilirubin levels at 1 year. In case of missing bilirubin at baseline or 1 year, the imputed laboratory data was used. Multiple imputation with by the Markov chain Monte Carlo method for missing data and Rubin's rules were used to estimate bilirubin and its standard error[14]. Ten imputed datasets based on the assumption that data were missing at random were created from iterations to reduce sampling variability.

The pattern of bilirubin (mean and 95% CI) over the first 5 years was evaluated in patients with normal bilirubin at time zero and stratified based on whether they experienced a late clinical event (LT or death from 5-10 years) or no clinical event in the first 10 years of follow-up. All patients included in the latter group had 10 years of follow-up. The imputed dataset was used for this analysis. A *P*-value less than 0.05 was considered statistically significant. All analyses were two-sided and were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Study population characteristics

A total of 3060 patients with normal bilirubin at baseline or one year after study entry were included. Two normal bilirubin cohorts were constructed based on the time point(s) at which their bilirubin levels were normal: time-zero cohort (n=2281) and 1-year cohort (n=2555). An overlap of 1821 patients exists between these cohorts. There were 297 and 344 primary endpoints according to each respective cohort. Patient characteristics per cohort are presented in **Table 1**.

Normal bilirubin quartiles are associated with survival

The quartiles in each individual cohort were formulated according to the following bilirubin levels (median [IQR], ×ULN): 0.53 (0.40-0.70) and 0.50 (0.38-0.67), respectively. In Kaplan-Meier analysis of patients that had normal bilirubin at time zero, the cumulative 10-year survival rate decreased with higher bilirubin quartiles and was 93.3%, 89.9%, 87.7%, 81.3% from quartiles 1-4 (Q1-Q4), respectively (**Figure 1**). In pairwise comparisons, Q4 was significantly different from Q1-Q3 (all $P < 0.005$). Additionally, Q1 was significantly different from Q3 ($P = 0.041$). Similar results were obtained in the Kaplan-Meier analysis of the 1-year cohort, in which the 10-year survival rates with increasing bilirubin quartiles were 92.0%, 92.3%, 86.1%, and 78.2%. Q3 and Q4 were significantly different from one another and from the remaining quartiles (all $P < 0.01$). In multivariable Cox regression analyses, normal bilirubin quartiles were a significant predictor for survival. In the time zero cohort, the risk for LT or death increased with higher bilirubin quartiles: Q1 (reference), Q2 (HR 1.12, 95% CI 0.73-1.72, $P = 0.61$), Q3 (HR 1.34, 95% CI 0.89-2.01, $P = 0.16$), Q4 (HR 1.83, 95% CI 1.24-2.71, $P = 0.003$). A similar trend was observed in the 1-year cohort: Q1 (reference), Q2 (HR 0.97, 95% CI 0.65-1.45, $P = 0.88$), Q3 (HR 1.46, 95% CI 1.02-2.10, $P = 0.04$), Q4 (HR 2.20, 95% CI 1.56-3.10, $P < 0.0001$).

Bilirubin threshold within the normal range to predict survival

Upon exploration of the optimal threshold of bilirubin within the normal range at 1 year, all bilirubin thresholds (0.3-0.9×ULN) were significant predictors of survival in that patients with

Table 1. Characteristics of PBC patients in each normal bilirubin cohort

	Time zero cohort (n=2281)	1-year cohort (n=2555)
Follow-up time, y, median (IQR)	7.9 (4.3-12.7)	7.3 (3.7-11.5)
Age at study entry, mean ± SD	55.3 ± 12.0	54.6 ± 11.8
Female, no. (%)	2086 (91.5)	2354 (92.1)
AMA-positive, no. (%)	2036/2222 (91.6)	2273/2485 (91.5)
Year of diagnosis, median (range)	1998 (1961-2014)	1997 (1961-2013)
UDCA-treated, no. (%)	1979/2223 (89.0)	2345/2523 (92.9)
Laboratory parameters, median (IQR)		
Total bilirubin, ×ULN	0.53 (0.40-0.70)	0.50 (0.38-0.67)
ALP, ×ULN	1.99 (1.27-3.32)	1.26 (0.88-1.96)
Albumin, ×LLN	1.17 (1.09-1.26)	1.17 (1.09-1.26)
AST, ×ULN	1.30 (0.93-1.93)	0.87 (0.65-1.20)
ALT, ×ULN	1.51 (0.98-2.35)	0.83 (0.58-1.33)
Platelet count, 109/L	255 (207-308)	250 (202-304)
Bilirubin ULN (mg/dl), median (IQR)	1.1 (1.0-1.2)	1.17 (1.0-1.2)

Abbreviations: No., number; AMA, anti-mitochondrial antibodies; UDCA, ursodeoxycholic acid; ULN, upper limit of normal; ALP, alkaline phosphatase; LLN, lower limit of normal; AST, aspartate aminotransferase; ALT, alanine aminotransferase; IQR, interquartile range.

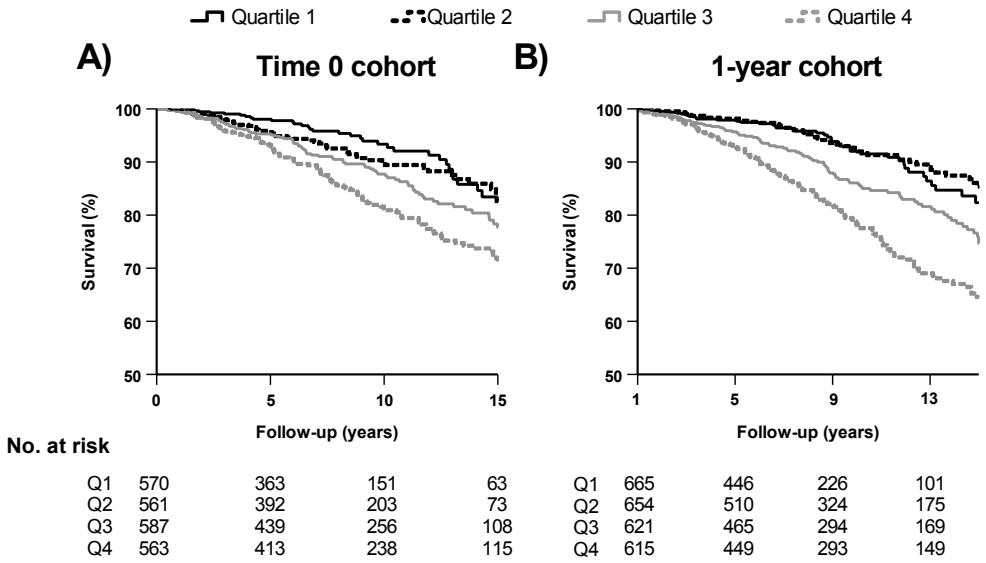

Figure 1. Survival estimates of bilirubin quartiles in patients with normal bilirubin at *A) time zero and B) 1 year.*

Table 2. Multivariable Cox regression analyses of various normal bilirubin thresholds at 1 year for the prediction of liver transplantation and death

Bilirubin at 1 year (n=2382)				
Threshold (×ULN)	**C-statistic (95%CI)**	**HR (95% CI)**	**P value**	**No. of patients ≤/> threshold**
0.30	0.7223 (0.6930-0.7515)	1.63 (1.04-2.58)	0.035	302/2080
0.40	0.7240 (0.6948-0.7531)	1.51 (1.12-2.06)	0.008	712/1670
0.50	0.7366 (0.7081-0.7651)	1.85 (1.46-2.36)	<0.0001	1243/1139
0.55	0.7357 (0.7073-0.7642)	1.90 (1.51-2.40)	<0.0001	1416/966
0.59	0.7400 (0.7114-0.7686)	2.02 (1.61-2.54)	<0.0001	1573/809
0.60	0.7429 (0.7144-0.7713)	2.12 (1.69-2.66)	<0.0001	1619/763
0.61	0.7423 (0.7137-0.7710)	2.09 (1.67-2.62)	<0.0001	1630/752
0.62	0.7385 (0.7095-0.7676)	2.00 (1.60-2.50)	<0.0001	1676/706
0.63	0.7385 (0.7095-0.7675)	2.02 (1.61-2.52)	<0.0001	1687/695
0.65	0.7351 (0.7061-0.7642)	1.89 (1.51-2.37)	<0.0001	1751/631
0.66	0.7354 (0.7063-0.7645)	1.90 (1.52-2.38)	<0.0001	1755/627
0.67	0.7361 (0.7070-0.7652)	1.92 (1.53-2.40)	<0.0001	1821/561
0.68	0.7354 (0.7064-0.7644)	1.89 (1.51-2.37)	<0.0001	1824/558
0.69	0.7341 (0.7051-0.7631)	1.88 (1.50-2.36)	<0.0001	1854/528
0.70	0.7344 (0.7055-0.7633)	1.91 (1.52-2.40)	<0.0001	1889/493
0.75	0.7346 (0.7052-0.7640)	1.96 (1.54-2.49)	<0.0001	1999/383
0.80	0.7336 (0.7045-0.7626)	2.14 (1.67-2.75)	<0.0001	2085/297
0.85	0.7291 (0.6997-0.7584)	1.89 (1.41-2.52)	<0.0001	2175/207
0.90	0.7253 (0.6959-0.7546)	1.86 (1.34-2.59)	<0.001	2242/140

bilirubin above each threshold had an increased risk for LT or death (**Table 2**). The bilirubin threshold at 1 year with the highest ability to predict LT or death was 0.6×ULN (C-statistic 0.7429, 95% CI 0.7144-0.7713). The 10-year survival of patients with normal bilirubin ≤0.6×ULN, normal bilirubin >0.6×ULN, and abnormal bilirubin 1 year were 91.3%, 79.2%, and 37.3%, respectively ($P < 0.0001$) (**Figure 2A**). At baseline, the 10-year survival rates were 91.7%, 85.6%, and 49.5% ($P < 0.0001$). We evaluated the distribution of clinical events from the 10-year survival rates associated with each bilirubin group. Clinical events in patients with bilirubin from 0.6-1.0×ULN were characterized by an increased proportion of LT and liver-related deaths, alongside a decreased proportion of liver-unrelated deaths compared to patients with bilirubin ≤0.6×ULN (**Supplementary Figure 1**). In an analysis of UDCA-treated patients with normal bilirubin levels >0.6×ULN at baseline (n=1170), a reduction in bilirubin ≤0.6×ULN at 1 year was associated with prolonged survival as compared to stable bilirubin that remained above the threshold and abnormal bilirubin after 1 year (both P <0.0001) (**Figure 2B**). The 10-year survival rate of these patients was 93%.

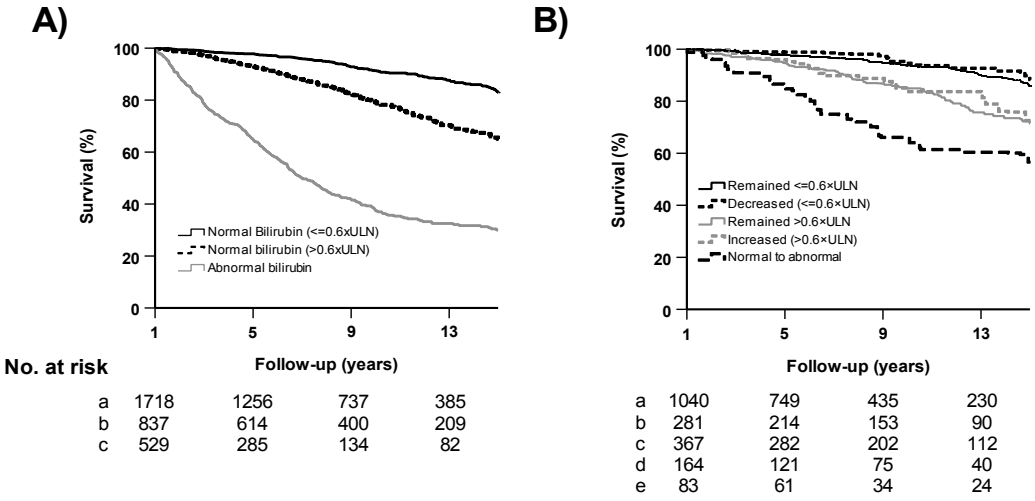

Figure 2. Survival estimates in patients with normal bilirubin (stratified by 0.6×ULN threshold) and abnormal bilirubin. *A) Kaplan-Meier estimates of survival rates in patients with normal bilirubin (stratified by 0.6×ULN threshold) and abnormal bilirubin at 1 year. B) Additional analysis of the survival rates in UDCA-treated patients with bilirubin levels >0.6×ULN at baseline.*

The threshold was evaluated in various sub-groups of patients that had normal bilirubin at 1 year, all of which confirmed that patients with bilirubin ≤0.6×ULN have a decreased risk for LT or death (**Figure 3**). Importantly, the association with a reduced risk remained when all patients with normal bilirubin in which the ULN was defined as ≥1.2mg/dL were excluded from the analyses (HR 2.10, 95% CI 1.54-2.85, $P < 0.0001$). Although the threshold of 0.6×ULN did not reach statistical significance in males and those with a histological stage III-IV, bilirubin levels above the threshold were also associated with an increased risk in these patients.

The risk for liver transplantation or death increases at bilirubin levels of 0.6×ULN

We assessed bilirubin on a continuous spectrum with a restricted spline function to evaluate whether the predetermined threshold is the point at which the hazard ratio for LT or death increases. The reference in each cohort was the predetermined threshold of 0.6×ULN. In both cohorts, the risk for LT or death remained stable below 0.6×ULN (**Figure 4**). However, beyond this threshold, a linear relationship was observed between bilirubin and the risk for LT or death that continued past the normal range. The test for curvature, which establishes whether there is a significant deviation from a linear relationship, was significantly different for the time zero ($P = 0.03$) and 1-year cohort ($P = 0.05$). As a sensitivity analysis, the restricted spline function analysis was repeated using crude bilirubin levels (mg/dL) (**Supplementary Figure 2**). The spline function analyses were also repeated with normal bilirubin levels at other time points (2-5 years) (**Supplementary Figure 3**).

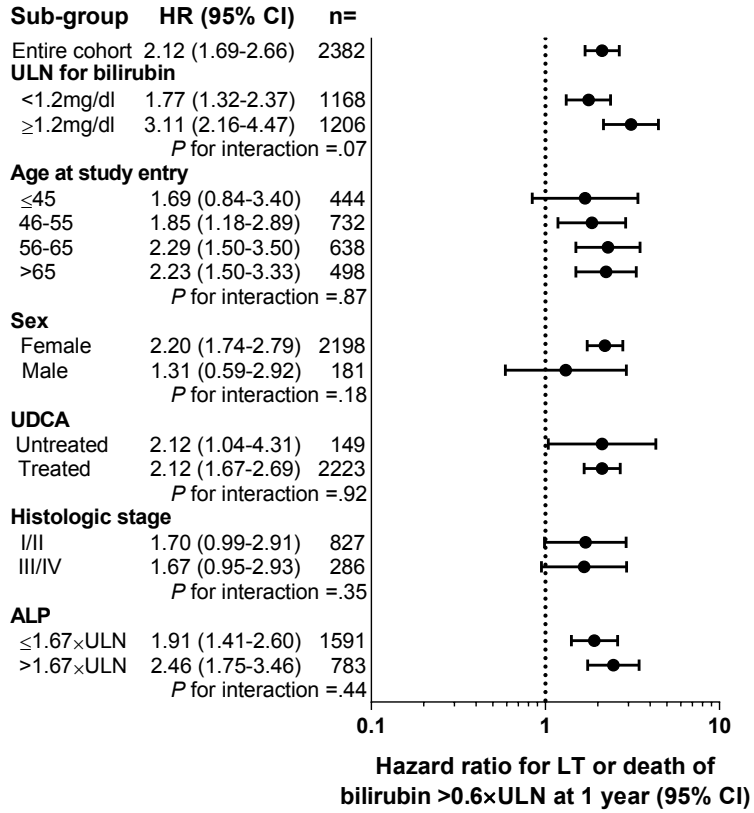

Figure 3. Sub-group analyses based on the bilirubin threshold of 0.6×ULN in patients with normal bilirubin at 1 year. Hazard ratio for liver transplantation or death (95% CI) obtained from multivariable Cox regression analyses in patients with normal bilirubin in various sub-groups. The hazard ratios correspond to bilirubin levels >0.6×ULN (versus bilirubin ≤0.6×ULN).

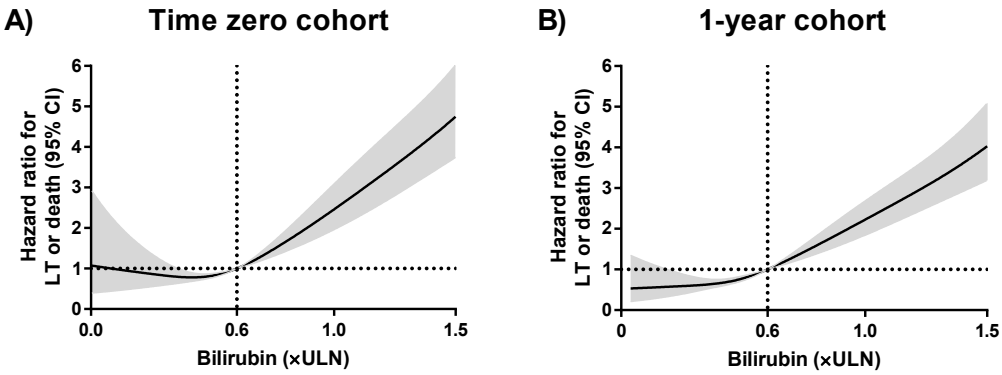

Figure 4. The association between bilirubin levels (×ULN) and risk for liver transplantation or death. Hazard ratios and 95% CI were estimated by a restricted cubic spline function in A) the time zero cohort and B) the 1-year cohort. The bilirubin reference in each cohort is 0.6×ULN.

Patients who remain below 0.6×ULN over time have good long-term prognosis

To assess how the trajectory of bilirubin over time may be related with the development of a clinical event (LT or death), bilirubin levels over the course of 5 years were evaluated in patients with normal bilirubin at time zero. The patients were stratified according to whether they developed a late clinical event from 5-10 years (n=103) or did not develop a clinical event in the first 10 years of follow-up (n=848). Patients who had no clinical event within 10 years of follow-up presented with a mean bilirubin level of 0.55×ULN (95% CI 0.54-0.56) and demonstrated stable bilirubin levels (below 0.6×ULN) in the first five years (**Figure 5**). In contrast, patients who reached a clinical endpoint presented with slightly higher mean bilirubin levels (0.61×ULN, 95% CI 0.57-0.65, *P* = 0.01) and exhibited a gradual increase within the normal range that precluded the occurrence LT or death.

ALP levels below 1.67×ULN are associated with transplant-free survival

In a subgroup analysis of patients with ALP ≤1.67×ULN from the normal bilirubin cohort at 1 year (n=1523), the optimal ALP threshold was 1.0×ULN (C-statistic, 95% CI). The HR for LT or death was 1.44 in those with ALP>1×ULN (95% CI 1.04-2.00, *P* = 0.03). Patients with ALP ≤1×ULN had the highest survival rate at 10 years (93.2%), compared to those with ALP between 1.0-1.67×ULN (86.1%), and ALP>1.67×ULN (85.4%) *P* < 0.005. Interestingly, the survival rate of the ALP 1.0-1.67×ULN group was not significantly different from that of ALP 1.67-3.0×ULN (*P* = 0.64) (**Supplementary Figure 5**).

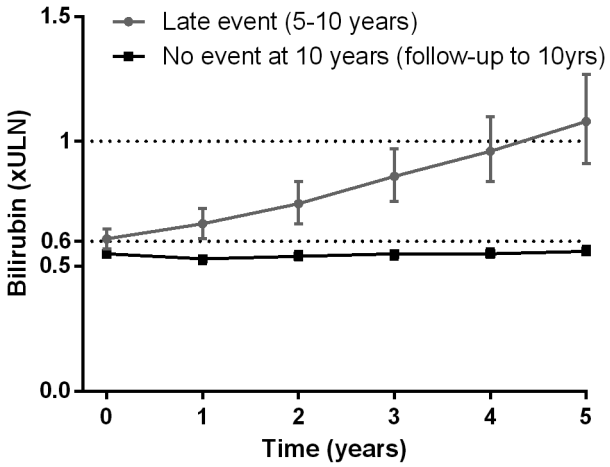

Figure 5. Mean bilirubin levels over 5 years in patients with normal bilirubin at study entry and stratified by outcome. Trajectory of the mean bilirubin levels (×ULN) and 95% CI over the first 5 years depending on whether they experienced a late clinical event between 5 and 10 years (n=132) or no event within the first 10 years of follow-up (n=979). Clinical event is defined as liver transplantation or death. All patients without a clinical event had a follow-up of at least 10 years.

Alkaline phosphatase normalization and bilirubin levels below 0.6xULN

Implementing both ALP and bilirubin thresholds established, the prognosis of patients with 282 bilirubin >0.6xULN was dependent on ALP normalization (**Supplementary Figure 5**). Given normal ALP levels, their survival rates were similar to those with bilirubin ≤0.6xULN, however if ALP was 1.0-1.67xULN, their survival was diminished to 74.2% at 10 years and 63.4% at 15 years (P<.001 compared to remaining groups).

DISCUSSION

This study reports that bilirubin levels within the normal range are associated with the risk for LT or death in patients with PBC. We demonstrated that bilirubin levels ≤0.6×ULN at baseline and 1 year were associated with a decreased risk for LT or death compared to patients with bilirubin above this threshold and that a reduction in bilirubin within the ULN after 1 year of UDCA therapy was associated with prolonged survival. While the risk for LT or death was stable when bilirubin levels were below 0.6×ULN, beyond this threshold, a positive linear relationship was observed between bilirubin and the risk for a clinical event. These results were confirmed in several sub-groups of patients. Our findings suggest that the interpretation of not being at risk if bilirubin is within the normal range needs to be revised. Additionally, ALP levels below 1.67×ULN were also associated with survival. This might have implications in the number of patients eligible for inclusion in clinical trials that assess novel second-line therapies since ALP levels >1.67×ULN/abnormal bilirubin are eligibility requirements that have been previously implemented.

Although previous studies reported that the ULN of bilirubin was the most predictive for survival in patients with PBC and considered a reasonable threshold[2], we found that the risk for LT or death is already increased when bilirubin levels were above 0.6×ULN. Similarly, the optimum bilirubin cut-off associated with survival has been previously identified to be lower than the ULN[15]. The current ULN of bilirubin represents the 97.5 percentile cut-off in the general population, yet this may not be the best approach to determine an optimal threshold since levels below this threshold are not reflective of an absence of increased risk[16]. In part, this might be explained by the high percentage of individuals with Gilbert's syndrome in the general population, which ranges from 3-10%[17]. Additionally, the current ULN of bilirubin may be a suboptimal threshold for risk stratification in PBC due to the female predominance of the disease, while sex differences in bilirubin are present in the general population[18]. An American study based on the Third National Health and Nutrition Examination Survey (NHANES III) assessed serum bilirubin levels in 16,865 adults from the general population and reported that mean serum bilirubin levels are significantly lower in women (0.52 mg/dL ± 0.003) than men (0.72 mg/dL ± 0.004)[16]. Consequently, the 97.5 percentile cut-off was 0.5 mg/dL higher in men. Other studies have reported similar sex

differences in bilirubin levels in the general population[19,20]. Thus, the overall ULN of bilirubin may be skewed to higher levels in PBC because of the inclusion of both men and women. These considerations suggest that the ULN for bilirubin may need to be stratified by sex, as has been previously implemented for aspartate aminotransferase[21,22].

We found that the predictive value of the bilirubin threshold of 0.6×ULN was irrespective of age, treatment with UDCA, and ALP levels. Importantly, it remained significantly predictive at various independent time points. Furthermore, in patients treated with UDCA that had a bilirubin level above 0.6×ULN but below the ULN at initiation of treatment, we found that a reduction below 0.6 was associated with significantly prolonged survival as compared to remaining within the normal range or increasing to an abnormal bilirubin level. This suggests that besides the predictive value of bilirubin within the normal range, a treatment-induced reduction of bilirubin within the current normal range is beneficial for long-term prognosis, which could have important implications for current patient care, but also for the design and interpretation of future clinical trials of potential second-line therapies in PBC. While recent clinical trials have often included normalization of bilirubin as a primary endpoint, it might be preferable to aim for lower bilirubin levels[23,24].

The pattern of bilirubin within the current normal range over time may also be relevant, as there was an overall increase of 0.47×ULN in mean bilirubin during the first 5 years of follow-up in patients who eventually reached a clinical endpoint after extended follow-up. While rapid increases in bilirubin have been shown to preclude death in untreated patients, these results suggest that there is an association between the trajectory of bilirubin and clinical outcomes even if within the normal range[3].The fact that the mean bilirubin levels of patients who did not experience a clinical event remained below 0.6×ULN over time further support an incentive to aim for bilirubin levels below our proposed threshold of 0.6×ULN. Further, our findings emphasize the importance of the continuous clinical evaluation of patients' bilirubin levels even in those with early stage disease.

A robust analysis of the predictive value of bilirubin within the normal range would not be possible without the large number of patients and extended follow-up available from the Global PBC Study Group cohort. Furthermore, bilirubin was assessed at multiple independent time points to confirm that bilirubin levels obtained during a random follow-up assessment could also be utilized for risk stratification. Nonetheless, some study limitations should be noted. Whereas, total serum bilirubin levels in healthy patients are primarily composed of unconjugated bilirubin, total bilirubin in patients with PBC is predominantly of conjugated form that leaks into the serum when it is unable to be excreted through bile[25]. However, bilirubin was available as total bilirubin in this study due to the fact that independent measurement of the conjugated and unconjugated forms is not part of routine standard of care in the majority of laboratories.

Although bilirubin was analyzed based on the ULN defined by local centers, which ranged from 0.6-1.7mg/dL, sensitivity analyses were performed to address this. The analyses with crude bilirubin levels (mg/dL) as well as the one excluding patients with an ULN above 1.2mg/dL confirmed our initial findings and exclude the possibility that patients with bilirubin levels above 0.6×ULN have worse survival due to the utilization of high ULNs.

In this multi-center international follow-up study of patients with PBC, bilirubin levels below the current ULN were shown to be predictive of survival and 0.6×ULN was established as the threshold from which point on the risk for LT or death increases. Additionally, reduction within the current normal range to below 0.6×ULN was associated with prolonged survival. Our proposed threshold of 0.6× the current ULN of bilirubin may be a more sensitive reference to identify patients at risk for a poor outcome and represent a threshold that increases the number of patients included in intervention studies that may benefit from therapeutic agents.

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SUPPLEMENTARY DATA

Supplementary Table 1. Survival estimates at 15 years according to bilirubin quartiles and bilirubin threshold of 0.6×ULN

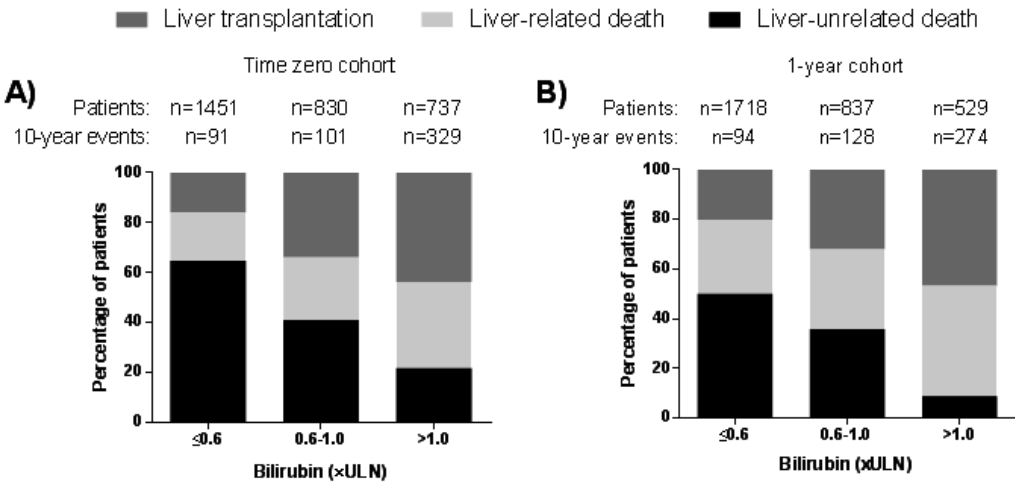
	15-year survival rates (%)	
Bilirubin quartiles	**Time zero cohort**	**1-year cohort**
Q1	83.4	82.3
Q2	82.6	85.4
Q3	77.6	74.6
Q4	71.3	64.6
Bilirubin threshold	**Time zero cohort**	**1-year cohort**
<0.6×ULN	82.0	82.9
0.6-1.0×ULN	73.0	65.1
>1×ULN	33.4	30.0

Abbreviations: ULN, upper limit of normal.

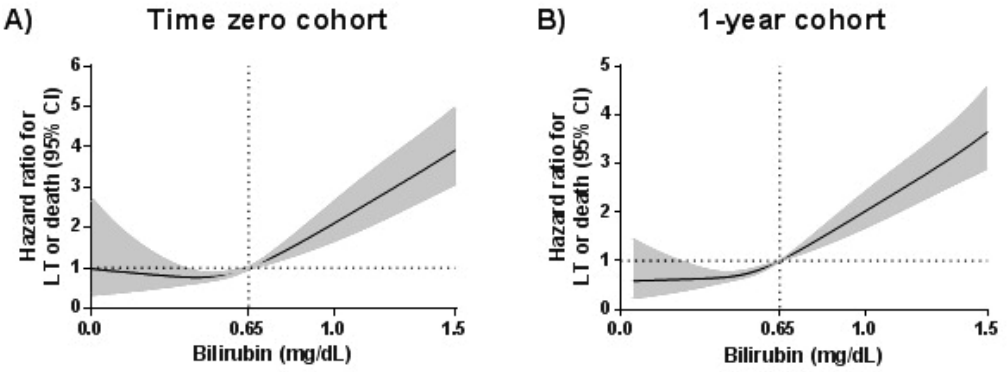
Supplementary Table 2. Multivariable Cox regression analyses of various normal bilirubin thresholds at time zero for the prediction of liver transplantation and death.

Threshold (×ULN)	C-statistic (95% CI)	HR (95% CI)	vvalue	No. of patients ≤/> threshold
0.30	0.734 (0.702-0.765)	1.48 (0.90-2.43)	0.13	228/1824
0.40	0.736 (0.705-0.767)	1.43 (1.00-2.05)	0.053	522/1530
0.50	0.740 (0.710-0.771)	1.56 (1.19-2.05)	0.001	979/1073
0.55	0.738 (0.707-0.769)	1.44 (1.11-1.86)	0.006	1135/917
0.60	0.740 (0.709-0.771)	1.47 (1.15-1.89)	0.002	1323/729
0.65	0.747 (0.716-0.777)	1.71 (1.33-2.19)	<0.0001	1435/617
0.66	0.747 (0.717-0.778)	1.70 (1.33-2.17)	<0.0001	1439/613
0.67	0.745 (0.715-0.776)	1.59 (1.24-2.03)	<0.001	1492/560
0.68	0.742 (0.712-0.773)	1.52 (1.18-1.95)	0.001	1503/549
0.69	0.742 (0.712-0.773)	1.52 (1.18-1.96)	0.001	1527/525
0.70	0.743 (0.712-0.773)	1.54 (1.20-1.98)	0.001	1551/501
0.75	0.741 (0.711-0.772)	1.60 (1.22-2.08)	0.001	1681/371
0.80	0.740 (0.709-0.771)	1.57 (1.18-2.09)	0.002	1769/283
0.85	0.740 (0.709-0.771)	1.66 (1.22-2.27)	0.001	1860/192
0.90	0.743 (0.713-0.774)	2.17 (1.53-3.09)	<0.0001	1929/123

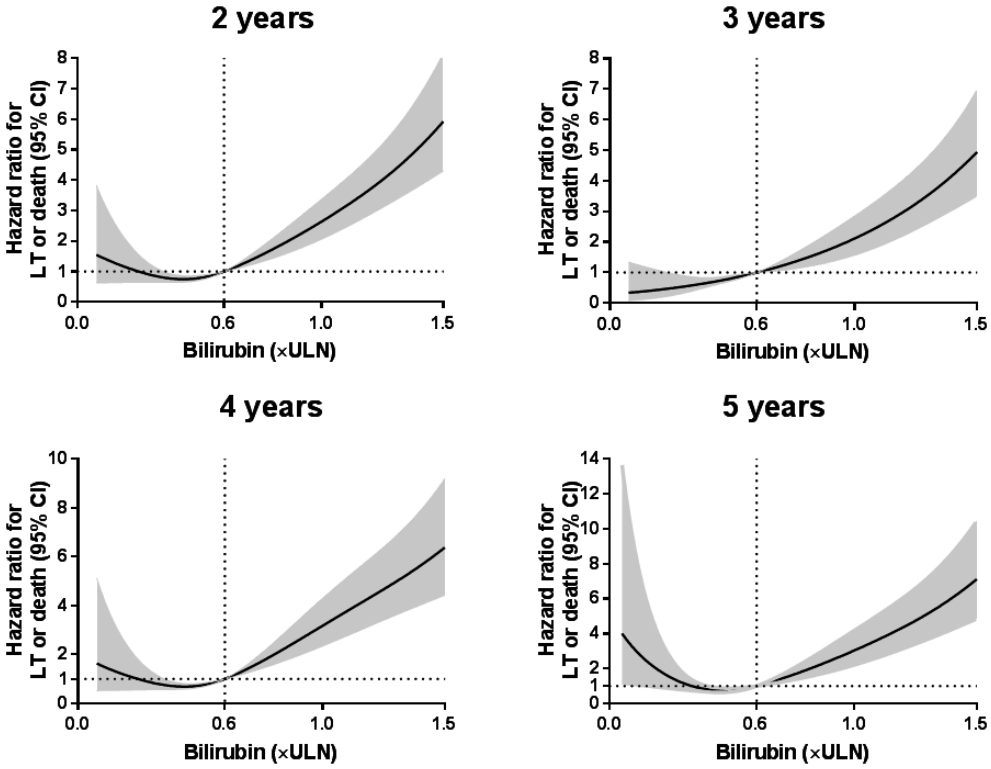
Abbreviations: ULN, upper limit of normal; HR, hazard ratio; CI, confidence interval.



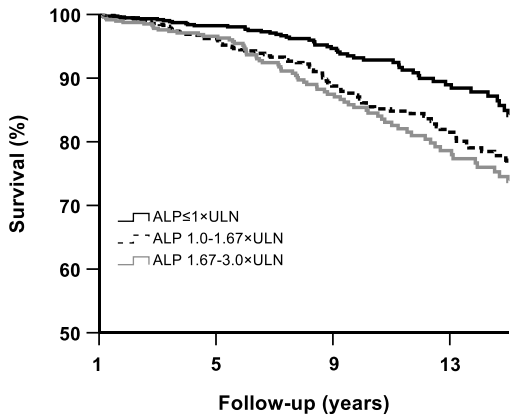
Supplementary Fig. 1. Distribution of clinical events from the 10-year survival rates associated with each bilirubin group. Distribution of liver transplantation, liver-related death, and liver-unrelated death at (A) time zero and (B) 1 year. There was a significantly different distribution in the type of event according to bilirubin group at baseline and 1 year: $P < 0.001$ (Pearson Chi-square).



Supplementary Fig. 2. The association between bilirubin levels (mg/dL) and risk for liver transplantation or death. Hazard ratios and 95% CI were estimated by a restricted cubic spline function in (A) the time zero cohort and (B) the 1-year cohort. The bilirubin reference in each cohort is 0.65 mg/dL.

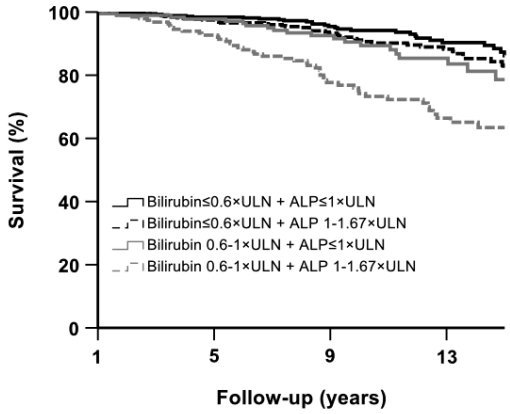


Supplementary Fig. 3. The association between bilirubin level and risk for liver transplantation or death from 2-5 years. Hazard ratios and 95% CI were estimated by a restricted cubic spline function at 2-5 years. The bilirubin reference in each cohort is 0.6×ULN.



a	773	555	340	171
b	750	559	335	177
c	482	354	218	124

Supplementary Fig. 4. Survival estimates stratified by ALP levels in patients with normal bilirubin at 1 year. Survival rates were significantly different between ALP≤1×ULN and 1.0-1.67×ULN (P = 0.001) and 1.67-3.0×ULN (*P* < 0.001), yet there was no significant difference between ALP 1.0-1.67 and ALP 1.67-3.0×ULN (*P* = 0.64). Survival was compared with the log-rank test.



a	579	407	243	123
b	552	416	238	126
c	194	148	97	48
d	198	143	97	51

Supplementary Fig. 5. Survival estimates according to the established thresholds for bilirubin and alkaline phosphatase. Patients with normal bilirubin (≤1×ULN) and ALP≤1.67×ULN at 1 years were included.

Harms MH, Lammers WJ, Thorburn D, Corpechot C, Invernizzi P, Janssen HLA, Battezzati PM, Nevens F, Lindor KD, Floreani A, Ponsioen CY, Mayo MJ, Dalekos GN, Bruns T, Pares A, Mason AL, Verhelst X, Kowdley KV, Goet JC, Hirschfield GM, Hansen BE, Van Buuren HR, Global PBC Study Group. Am J Gastroenterol. 2018 Feb;113(2):254-264.

CHAPTER 3
MAJOR HEPATIC COMPLICATIONS IN URSODEOXYCHOLIC ACID-TREATED PATIENTS WITH PRIMARY BILIARY CHOLANGITIS: RISK FACTORS AND TIME TRENDS IN INCIDENCE AND OUTCOME

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ABSTRACT

Objectives In this era of near universal ursodeoxycholic acid (UDCA) treatment for primary biliary cholangitis (PBC), progression to cirrhosis still occurs in an important proportion of patients. The aim of this study was to describe the incidence of cirrhosis-associated complications in patients with PBC and assess risk factors and impact on survival.

Methods Cohorts of UDCA -treated patients from 16 European and North-American liver centers were included. We used Cox proportional hazards assumptions and Kaplan–Meier estimates.

Results During 8.1 years' median follow-up, 278 of 3,224 patients developed ascites, variceal bleeding, and/or encephalopathy (incidence rate of 9.7 cases/1,000 patient years). The overall cumulative incidence was 9.1% after 10 years of follow-up, but decreased over time to 5.8% after the year 2000. Earlier calendar year of diagnosis ($P < 0.001$), high aspartate aminotransferase to platelets ratio index (APRI; $P < 0.001$) and biochemical non-response ($P < 0.001$) were independently associated with future complications. Patients with both biochemical non-response and an APRI >0.54 after 12 months of UDCA had a 10-year complication rate of 37.4%, as compared to 3.2% in biochemical responders with an APRI ≤0.54. The 10-year transplantation-free survival after a complication was 9% (time-dependent hazard ratio 21.5; 20.1–22.8). Prognosis after variceal bleeding has improved over time.

Conclusions In this large international cohort, up to 15% of UDCA-treated PBC patients developed major non-neoplastic, cirrhosis-associated hepatic complications within 15 years, but cumulative incidence has decreased over time. Biochemical non-response to UDCA and APRI were independent risk factors for these complications. Subsequent long-term outcome after complications is generally poor, but has improved over the past decades.

INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic cholestatic autoimmune disease, characterized by inflammatory destruction and loss of intrahepatic bile ducts. The disease usually progresses slowly and may eventually lead to cirrhosis and its associated complications[1, 2].

While the standard of care treatment ursodeoxycholic acid (UDCA)[2-4] has been found to improve survival[5-7], twenty to fifty percent of patients respond sub-optimally to this treatment[8-13]. Studies over the past decades suggest that nowadays only a minority of approximately 15% of patients have evidence of advanced disease at presentation[12, 14-16] and that UDCA treatment is associated with a much slower histological disease progression than when patients are left untreated[17-19]. However, the cumulative incidence of cirrhosis over a 10-year follow-up in a recent large cohort study was approximately 40%[14]. This indicates that a substantial proportion of PBC patients might still face a risk of cirrhosis-associated complications at some point during their disease.

Cirrhosis-associated complications are likely to be negatively correlated with prognosis[20, 21]. Few studies have specifically evaluated the incidence of and risk factors for major cirrhosis-associated complications such as ascites, encephalopathy and gastrointestinal bleeding in patients with PBC[22-26]. These studies either included small populations or a large majority of untreated patients, or did not provide detailed information regarding the spectrum of potential complications and the associated outcome. In light of the near universal treatment with UDCA for PBC today, better knowledge of the risk and impact of major cirrhosis-associated complications in a UDCA-treated population of patients with PBC is important and could be helpful for patient counselling and therapeutic decisions.

In this study we aimed to assess the incidence and risk factors for major, non-neoplastic hepatic complications in a large, internationally representative cohort of UDCA-treated PBC patients. Secondly, we aimed to assess potential differential effects of such events on long-term prognosis. Finally we were interested to assess potential changes over time in the incidence and the prognostic impact of these complications.

PATIENTS AND METHODS

Population and study design

For the current study, patient data from 15 liver units across 10 countries in Europe and Northern America that are engaged in the Global PBC Study Group, were included. Individual patient data from long term follow-up cohorts were combined. In the individual participating centers, most data were collected prospectively, usually over several decades. To ensure a uniform database, additional data were collected retrospectively when indicated. Only UDCA-treated patients with an established diagnosis of PBC in accordance with internationally accepted guidelines[2, 3] were eligible for inclusion in this study. Individuals were excluded from analysis in case of insufficient follow up data (<6 months follow up or <2 visits recorded), when dates of starting treatment or clinical events were unknown and when PBC-autoimmune hepatitis overlap syndrome[3] or other concomitant liver disease was present. To ensure reliable risk factor analysis, occurrence of an hepatic complication within the first year of follow up or prior to the start of UDCA therapy, was also reason to omit patients from analysis.

Diligent efforts, including individual center visits to review the historical medical charts, were made to ensure data completeness. This study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the institutional research board of the corresponding center and at all participating centres as per local regulations.

Data collection

Baseline was set at the moment of starting UDCA therapy. The following clinical and biochemical data was collected: date of birth, sex, date of PBC diagnosis, liver biopsy, treatment (type, dosage, duration), baseline anti mitochondrial antibody (AMA) status, baseline and yearly biochemistry (serum alkaline phosphatase (ALP), total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, γ-glutamyl transpeptidase (g-GT), platelets) and clinical outcomes (death, liver transplantation, hepatocellular carcinoma, ascites, variceal bleeding, hepatic encephalopathy).

Histological baseline information was taken into account when a liver biopsy was available within one year from baseline. Histological disease severity was classified according to the Ludwig and Scheuer classification[27]. Stage I and II were considered to be early stage disease. Stage III and IV were classified as advanced disease. Biochemically early versus advanced disease was classified according to serum bilirubin and albumin levels[28].

Patients were followed according to the standard follow-up visit scheme of the participating centres. This usually included a clinic visit every 12 months for early disease stages and every 6 months for patients with advanced disease. Only those Global PBC Study Group centres for which data on complications were available participated in this study. Data of the original

cohorts were collected to 31 December 2012[29]. For three centres more recently joining the study group, data were collected up to 31 December 2015 (**Supplementary Table 1**).

Hepatic complications

Data regarding the following cirrhosis-associated complications were collected: gastrointestinal variceal bleeding, ascites and hepatic encephalopathy, whichever came first. Ascites was deemed to be present when ascitic fluid was confirmed by abdominal imaging or in the event of prescribed diuretic treatment for clinically obvious ascites. A diagnosis of hepatic encephalopathy was based on expert (physician) opinion and was scored present or not present. Variceal bleeding was defined as hematemesis or melena due to endoscopically documented hemorrhage originating from gastro-esophageal varices.

Statistical analysis

The primary outcome of this study was defined as the first occurrence of variceal bleeding, ascites or hepatic encephalopathy. Patients without these complications were censored at time of last follow-up, liver transplantation or death. For survival analyses, a combined endpoint of liver transplantation and death was used. The significance of biochemical response to UDCA was analyzed using the Barcelona, Paris, Rotterdam and Toronto biochemical criteria, and the recently published GLOBE and UK-PBC scores[8-11, 13, 30, 31]. The risk of complications was also analysed according to the AST to platelet ratio index (APRI) at 1 year of follow-up, which was also dichotomized according to the cut-point proposed by *Trivedi et al*[16]. Univariate and multivariable Cox proportional hazards models were fit to assess risk factors of patient characteristics and laboratory covariates on hepatic complications, and to assess covariates at time of a major hepatic complication that were associated with liver transplantation or death. In these analyses, we deliberately chose to not include histological disease stage as a variable, given there was no histological information for nearly 40% of the study population. A clock-reset approach was used to visualize the impact of a first hepatic complication on survival. The effect was assessed by time-dependent Cox regression analyses. In this approach patients who had a major hepatic complication were switched to a new survival curve which was then reset as time 0 for their further follow-up[32]. To identify a subgroup of patients with a more favourable survival after occurrence of a major non-neoplastic hepatic complication, we first tested the prognostic value of variables in univariate Cox regression analyses. We therefore also dichotomized laboratory data at different cut-offs, based on clinical relevance and distribution in the study cohort. Subsequently, a multivariable model was constructed. Multivariable analyses were stratified by center to adjust for center-specific effects.

Data are presented as median and its interquartile range (IQR) for continuous variables when applicable. A value of $p<0.05$ was considered to be statistically significant. All analyses were conducted using IBM SPSS Statistics V.21.0 (Armonk, NY: IBM Corp.). Analyses to identify risk

factors for major hepatic complications were performed in a multiple imputed database. As a sensitivity analysis, a comparison with complete case analyses was provided.

SAS version 9.4 (SAS Institute Inc., Cary, NC) was used to generated 10 imputed datasets (to reduce sampling variability) of laboratory results at baseline and after one, two, three, four and five years of follow-up (SAS Proc MI, MCMC method)[33, 34]. We assumed missing data occurred at random. Rubin's rules were used for estimation of the parameters and the standard error[35, 36]. The imputation model variables included both those potentially predicting outcome and outcomes themselves. Only the continuous, biochemical values were imputed. The biochemical values included for imputation were: ALP, AST, ALT, total bilirubin, albumin and platelet count. In case of non-normality, the natural logarithm of these variables was used. No categorical or binary variables were imputed.

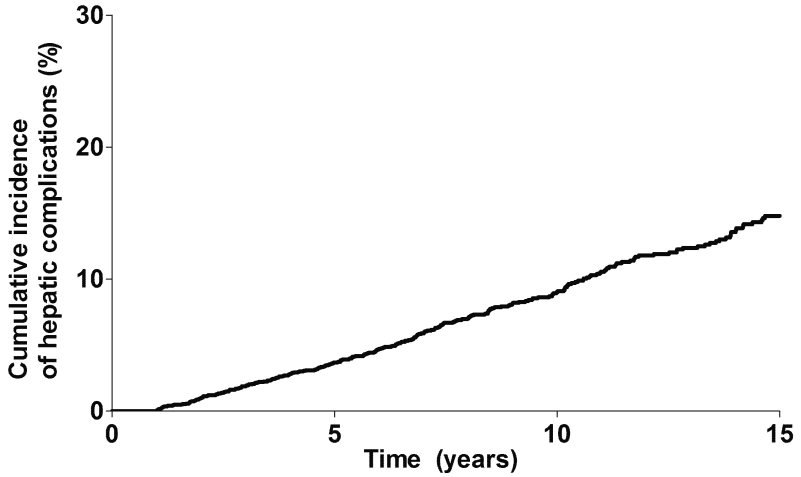
RESULTS

Study population characteristics

Of the 4294 UDCA-treated patients in our cohort, status of major hepatic complications was unknown for 959 patients. The study cohort comprised 3335 UDCA-treated patients with PBC. 111 patients with complications prior to or within the first year of follow up were excluded from analysis. The final population that was used for analyses included 3224 patients, of whom 91% were female and 73% had an early biochemical disease stage (**Table 1**). Median follow up until death, liver transplantation or censoring was 8.1 years (IQR 4.4-12.7) (**Supplementary Table 1**). During follow-up 150 patients underwent liver transplantation and 337 patients died (**Supplementary Figure 1**).

Incidence of complications

Hepatic complications were noted in 278 patients (**Figure 1**). The cumulative incidence rate of all first complications was 9.7 cases per 1000 patient-years. At 3, 5, 10, and 15 years of follow up, the complication rates in the study population were 1.9%, 3.7%, 9.1% and 14.8%, respectively (Figure 1). In a majority of 63% (n=175 patients), the first observed complication was ascites, as opposed to variceal bleeding in 23% (n=65 patients) and encephalopathy in 8% (n=22 patients). In a minority of 6% (n=16 patients) more than one type of complication was noted to occur at the same time. A sensitivity analyses in which patients that were diagnosed with HCC before the occurrence non-neoplastic major complications were censored at time of their HCC diagnosis, showed a very similar complication rate (**Supplementary Figure 2**). When the cumulative incidence was stratified according to the time-period of inclusion, we found that the cumulative incidence decreased over time, with a 10-year cumulative complication rate of 13.5% for patients included in or before 1990, 9.3% for patients included between 1990 and 2000, and 5.8% for patients included after



Patients at risk	3224	2256	1184	490
All complications	0	101	200	254
Acites	0	57	117	155
Variceal bleeding	0	34	55	64
Encephalopathy	0	9	20	25

Figure 1. Cumulative incidence of hepatic complications. For this analysis, only the first complication, either ascites, variceal bleeding or hepatic encephalopathy was taken into account.

the year 2000 (p<0.01 for all) (**Supplementary Figure 3**). Patients included in the later time periods more often had an early biochemical stage of disease at time of inclusion (75.8% and 74.9) than before or in 1990 (63.3%). Furthermore, the majority of patients included ≤1990 had an interval between diagnosis of PBC and initiation of UDCA therapy of at least two years (56.9%), as opposed to 30.4% of patients included between 1990 and 2000, and 9.4% of patients included after 2000 (**Supplementary Table 2**).

Risk factors for hepatic complications

First, we used univariate Cox regression analyses to identify covariates after 12 months of UDCA treatment that were associated with development of hepatic complications. In univariate Cox regression analyses, calendar year of diagnosis, years of untreated PBC diagnosis before starting UDCA therapy, higher AST/ALT ratio, higher APRI and biochemical non-response to UDCA according to the GLOBE score, were associated with an increased future risk of major hepatic complications. In multivariable analyses, calendar year of PBC diagnosis (per 10 years) (HR 0.73, 95% CI 0.60 - 0.89), a high AST / platelets ratio index (APRI) (HR 5.32, 95% CI 3.82-7.41) and biochemical non-response to UDCA based on the GLOBE score (comprising of age, ALP, bilirubin, albumin and platelet count) (HR 2.68, 95% CI 1.99-3.62) remained independently associated with the risk of major hepatic complications during follow-up (all p<0.01) (**Table 2**). A sensitivity analyses on complete cases showed similar results (**Supplementary Table 3**).

Table 1. Baseline cohort characteristics

	N = 3224
Age, years[a]	53.9 (11.8)
Female, n (%)	2937 (90.6)
AMA+, n (%)	2946 (91.4)
Year of diagnosis[b]	1997 (1990-2003)
Year of diagnosis, range	1968-2014
Year of enrolment[b,c]	1998 (1993-2004)
Year of enrolment, range	1977-2014
Histological disease stage, n (%)[c]	
Stage I	750 (37.8)
Stage II	650 (32.8)
Stage III	314 (15.8)
Stage IV	268 (13.5)
Biochemical disease staged, n (%)	
Bilirubin≤ULN and albumin≥LLN	1239 (73.2)
Bilirubin>ULN or albumin<LLN	381 (22.5)
Bilirubin>ULN and albumin<LLN	73 (4.3)
Serum total bilirubin ×ULN[b]	0.60 (0.43-0.93)
Serum ALP ×ULN[b]	2.17 (1.36-3.79)
Serum AST ×ULN[b]	1.50 (1.00-2.29)
Serum ALT ×ULN[b]	1.70 (1.05-2.72)
Serum albumin ×LLN[a]	1.16 (0.15)
Serum platelet count ×10*3/mm3*[a]	254 (88)
APRI[e]	0.59 (0.37-0.93)

Data on histological disease stage was missing for n=1241 (38.5%), biochemical disease stage was missing for n=1531 (47.5%,) total bilirubin was missing for n=946 (29.3%), ALP was missing for n=943 (29.1%), AST was missing for n=994 (30.7%), ALT was missing for n=1035 (32.9%), albumin was missing for n=1386 (43.0%), platelet count was missing for n=1428 (44.3%).

[a] Data is expressed as mean and standard deviation;

[b] Data is expressed as median and interquartile range;

[c] Equals the year of initiation of UDCA treatment;

[d] Histological stage according to Ludwig and Scheuer's classification[27];

[e] Biochemical disease stage was classified according to the Rotterdam criteria[28];

[f] AST/platelets Ratio Index[16, 54];

Abbreviations: AMA, anti-mitochondrial antibodies; ULN, upper limit of normal; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LLN, lower limit of normal; APRI, AST/platelets Ratio Index.

Table 2. Covariates associated with future development of hepatic complications after 12 months of ursodeoxycholic (UDCA) therapy.

	Univariate analysis			Multivariable analysis		
	HR	**95% CI**	**p**	**HR**	**95% CI**	**p**
Age *(per 10 years)*	1.08	0.97 - 1.21	0.176	-	-	-
Age < 40 years	0.74	0.50 - 1.10	0.140	-	-	-
Male sex	1.50	1.03 - 2.19	0.035	-	-	-
AMA negative	1.26	0.79 - 1.99	0.335	-	-	-
Year of diagnosis *(per 10)*	0.55	0.45 - 0.66	<0.001	0.73	0.60 - 0.89	0.002
Interval diagnosis - UDCA > 2 yrs[a]	2.01	1.55 - 2.61	<0.001	-	-	-
Advanced disease[b]	4.34	3.18 – 5.92	<0.001	-	-	-
Total bilirubin ×ULN[c]	3.70	3.11 - 4.41	<0.001	-	-	-
ALP ×ULN[c]	2.37	1.94 - 2.90	<0.001	-	-	-
AST ×ULN[c]	3.26	2.73 - 3.91	<0.001	-	-	-
ALT ×ULN[c]	2.07	1.74 - 2.46	<0.001	-	-	-
Albumin ×LLN	0.01	0.004 - 0.04	<0.001	-	-	-
Platelets *(per 50*x10^3/mm^3*)*	0.47	0.41 - 0.54	<0.001	-	-	-
AST/ALT ratio[c]	1.62	1.20 – 2.19	0.002	-	-	-
APRI[d], *continuous*[c]	2.85	2.59 - 2.75	<0.001	-	-	-
APRI> 0.54	6.97	5.11 - 9.50	<0.001	5.32	3.82 - 7.41	<0.001
UK-PBC score *(per 20)*[e]	1.53	1.40 - 1.63	<0.001	-	-	-
UK-PBC score ≤ median[e]	2.76	2.14 - 3.69	<0.001			
GLOBE-score, *continuous*	3.03	2.60 - 3.52	<0.001	-	-	-
Biochemical non-response[f]	5.52	4.17 - 7.33	<0.001	2.68	1.99 - 3.62	<0.001

[a] Years of untreated PBC diagnosis before starting UDCA therapy;
[b] Abnormal serum albumin and/or bilirubin[28];
[c] These variables were transformed with natural logarithm;
[d] AST/Platelets Ratio Index[16, 54];
[e] The median UK-PBC score in this study cohort was -103.3[31];
[f] Based on (age-dependent) GLOBE score thresholds[12];
Abbreviations: HR, hazard ratio; CI, confidence interval; AMA, anti-mitochondrial antibodies; ULN, upper limit of normal; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LLN, lower limit of normal; APRI, AST/Platelets Ratio Index.

Biochemical non-response and APRI score as predictors of hepatic complications

Both biochemical non-response after one year of UDCA and an APRI of >0.54 were independent predictors of future hepatic complications. The 10-year cumulative incidence of first complications was 32.4% in biochemical responders, as opposed to 6.2% in biochemical responders. In patients with an APRI of ≤0.54, the 10 year complication rate was 3.8%, compared to 24.3% in patients with a higher APRI. Patients with both an APRI of ≤0.54 as well as biochemical response after 12 months of UDCA, had 3, 5, and 10-year complication rates of 0.5%, 1.1% and 3.2% respectively. In contrast, patients with both an APRI >0.54 as well as biochemical non-response at 12 months had 3, 5- and 10-year complication rates of 15.9%, 22.1%, and 37.4%. In patients with either biochemical non-response and an APRI of ≤0.54, or biochemical response and an APRI of >0.54, the cumulative complication rates were found to be comparable to the overall cohort (**Figure 2**). Comparable predictive values for biochemical non-response were found when biochemical non-response was defined by other criteria (**Table 3**).

Table 3. Predictive value of response criteria for occurrence of hepatic complications

Response criteria	HR	95% CI	p value
GLOBE-score	6.046	4.773 - 7.660	<0.001
Paris-I	5.024	3.856 - 6.546	< 0.001
Paris-II	4.654	3.270 - 6.622	< 0.001
Rotterdam	4.397	3.295 - 5.866	< 0.001
Toronto	3.057	2.285 - 4.090	< 0.001
Barcelona	1.711	1.274 - 2.296	< 0.001

Biochemical response to UDCA was defined by previously reported criteria with a threshold defining response versus non-response, calculated by univariate Cox regression analysis. Criteria were calculated after 1 year of follow-up, Toronto criteria were calculated after 2 years of follow-up.
Abbreviations: HR, hazard ratio; CI, confidence interval.

Hepatic complications and subsequent survival

Overall transplantation-free survival at 1 year was 99.7%, at 3 years 97.3%, at 5 years 94.5% and at 10 years 85.8%. **Figure 3** visualizes the transplantation-free survival rate for patients with and without hepatic complications, estimated using a clock-reset approach[37]. For patients remaining free of complications the 3, 5, and 10 years transplantation-free survival rates were 97.1%, 94.2% and 85.3%, respectively. In contrast, after occurrence of a hepatic complication, these survival rates dropped to 34.7%, 19.2% and 10.4%, respectively (time-dependent HR 21.5; 20.1-22.8) (**Figure 3**). Transplantation-free survival after complications differed with respect to the nature of the first hepatic complication (p=0.004) (**Figure 4**). Median survival after occurrence of variceal bleeding as a first complication was 4.0

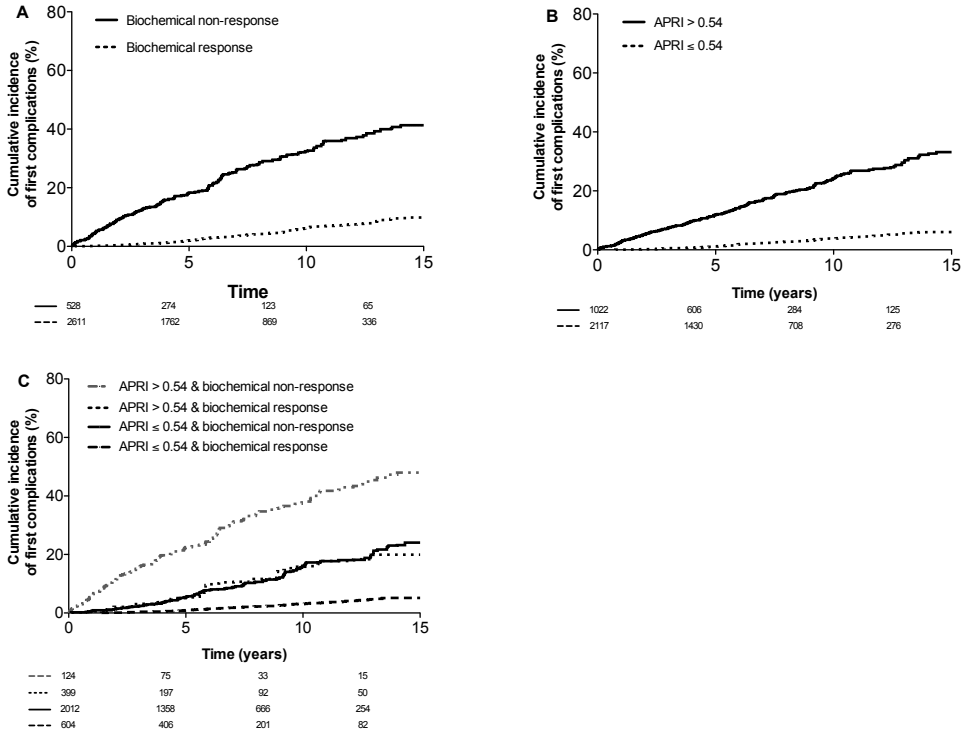


Figure 2. Cumulative incidence of hepatic complications according to biochemical response and APRI score. The GLOBE score and the APRI score were calculated after 12 months of follow-up. T=0 represents the time after 12 months of follow-up.

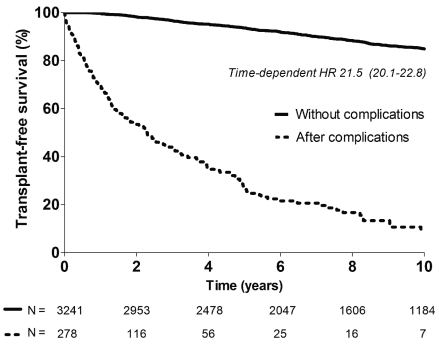


Figure 3. Transplantation-free survival related to development of hepatic complications. Visualisation of transplant-free survival according to occurrence of major hepatic complications, using a clock-reset approach. The solid line represents survival of all included patients (N=3241). The dotted line represents survival following occurrence of a hepatic complication (N=278).

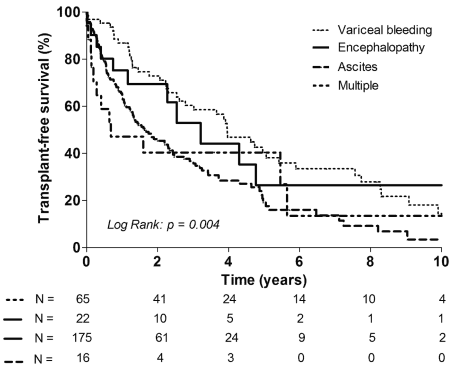


Figure 4. Transplant-free survival following the first occurrence of hepatic complications. Kaplan Meier estimates of transplant-free survival after the onset of a first hepatic complication (T_0) are shown. Patients either had a first episode of ascites (N=175), variceal bleeding (N=65), encephalopathy (N=22) or a combination of these (N=16).

years (95%CI 2.9-5.0), after occurrence of encephalopathy 3.2 years (95%CI 1.8-4.6), after occurrence of ascites 1.6 years (95%CI 1.0-2.3) and after multiple concurrent complications 0.6 years (95%CI 0.1-1.2). The difference in survival after occurrence of complications was significant for variceal bleeding as compared to ascites (p=0.001). No significant differences were found with respect to ascites versus encephalopathy (p=0.506) or variceal bleeding versus encephalopathy (p=0.232).

Prognostic factors at the time of complications

Several laboratory and clinical parameters at the moment of the first hepatic complication proved to be predictive of subsequent transplantation-free survival in univariate analyses. In multivariable Cox regression analysis, a lower calendar year of complications (p=0.022); serum albumin below the LLN (p<0.001); and serum bilirubin above the ULN (p<0.001) remained independently predictive of poor transplantation-free survival (**Supplementary Table 4**). When transplant-free survival after occurrence of a first major complication was stratified according to time period of occurrence of these complications, transplant-free survival complications was significantly shorter for patients that were affected earlier in calendar time (p=0.01), in particular after variceal bleeding (**Figure 5**). At the time of complications serum albumin and bilirubin levels were normal in 17% of the affected patients. With a median transplantation-free survival of 7.2 years (95%CI 5.1-9.3), prognosis of patients with normal serum albumin and bilirubin at time of their first hepatic complication was significantly better than the median 1.6 years (95%CI 1.2-1.9) survival of patients with abnormal serum albumin and/or bilirubin (p<0.001) (**Supplementary Figure 4**).

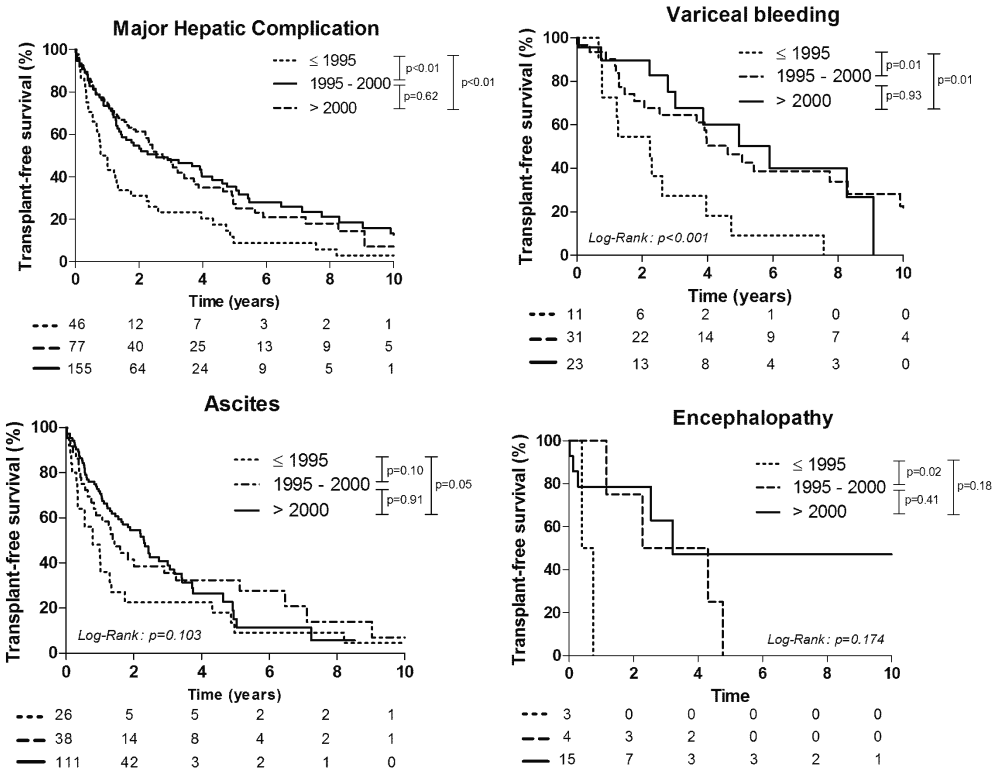


Figure 5. Transplant-free survival after first occurrence of a major hepatic complication stratified according to time period of patient inclusion. (A) a first major hepatic complication (B) ascites, (C) variceal bleeding and (D) encephalopathy.

DISCUSSION

In this long-term study of a large, internationally representative cohort of PBC patients treated with UDCA, the current standard therapy, we found that the overall cumulative incidence of major non-neoplastic hepatic complications was 9% after 10 years and 15% after 15 years. The cumulative incidence of these complications decreased over the past decades. Furthermore, in patients responding well to UDCA treatment and a low APRI after 12 months of treatment, the 10-year complication risk was reduced to only 3%, which is in marked contrast with a 10-year risk of 37% in patients with a high APRI and non-response to UDCA. Our data confirm that these complications are of critical importance in the course of the disease and are predictive of poor survival.

The decrease in cumulative incidence of major hepatic complications over time may be explained by a number of reasons. Firstly, our data shows that over the past decades, patients are increasingly being diagnosed in an early stage of disease[2]. Secondly, likely related to the gradual introduction of UDCA and increasing evidence for a therapeutic benefit of this agent in the last decades of the 20th century, we found that the interval between diagnosis and initiation of UDCA treatment strongly decreased over time. Also, other developments, such as timely detection and widespread primary prophylaxis of variceal bleeding, may have contributed to a decreasing incidence of complications[38]. In keeping with previous studies on specific complications, risk factors we found to be associated with future development of complications are largely the same as those related to transplant-free survival[8, 16, 22, 31, 39]. Similarly to baseline factors associated with future development of hepatocellular carcinoma[40] and with risk of overall mortality in PBC[30], biochemical non-response to UDCA therapy proved to be a strong risk factor for the occurrence of major hepatic complications during follow-up. This finding stresses the clinical importance of evaluating biochemical response to UDCA after one year of therapy. Interestingly, our data confirmed the additional prognostic value of APRI independent of biochemical response[16]. Stratification by both biochemical response and APRI identified a subgroup of approximately 20% of our cohort with a very low long-term risk of major, non-neoplastic hepatic complications.

As in other chronic liver disease, the development of hepatic complications is predictive for high mortality in PBC. In agreement with a systematic review assessing survival after occurrence of cirrhosis-associated complications across several liver diseases[41], median survival after the first complication in our cohort was approximately two years. The present study also shows that a subgroup of patients develop major hepatic complications in the absence of biochemical features of markedly advanced liver disease. The subsequent prognosis of these patients was considerably better than when liver function was more severely compromised. Our data furthermore indicate that the particular type of hepatic complication is predictive of subsequent survival. Interestingly, patients suffering from

variceal bleeding as the first major complication have a more favorable prognosis than patients who first develop ascites. The calendar year of occurrence of these events was an independent predictor of subsequent prognosis, with better outcomes for more recent cases, particularly for variceal bleeding. Improved patient care seems a likely explanation for this observation, which is in agreement with studies reporting improved prognosis after variceal bleeding over the last decades[42, 43].

Ascites is generally considered the most frequent first decompensating event in patients progressing from compensated to decompensated cirrhosis[44-47]. Our finding that ascites is also the most frequent first decompensating event in PBC is in agreement with the results of the classical long-term cohort study performed in Northeast England, reporting that ascites was both the most prevalent cirrhotic complication at the time of diagnosis as well as during follow-up[48]. Although the development of ascites or other complications is associated with poor prognosis, a subset of approximately 10% of our patients, particularly those with well-preserved hepatic function, was found to be alive without transplantation after 10 years. This indicates that major cirrhotic complications can occur relatively early in the course of PBC. Although portal hypertension due to nodular regenerative hyperplasia or other non-cirrhotic architectural histological changes has been reported in PBC[49-51] this seems a very rare entity and cirrhosis is the usual underlying cause of portal hypertension[21, 52]. More detailed characterization of the patients in our cohort with favorable long-term prognosis despite major hepatic complications was not the explicit aim of the current study but could be an interesting subject for future study.

Limitations of this study relate mostly to the nature of this study. Although the cumulative database includes prospectively followed-up patients, not all information relevant for this study, such as laboratory data at the time of hepatic events, was uniformly available in the individual data-sets. We were able to correct this by means of multiple imputations. Importantly, we do not have data on possible additional major hepatic complications after the first event. These data may provide an additional predictive value for survival and could be explored as such in the future. For practical reasons, this study did not address other, more rare complications of (cirrhotic) liver disease such as hepatopulmonary syndrome, portopulmonary hypertension, and cirrhotic cardiomyopathy. However, we speculate that it is unlikely that the main results of the present analyses would have been markedly different if we would have been able to do so. Our study group addressed hepatocellular carcinoma as a major potential complication of PBC in a previous large cohort study[40]. For this reason it was decided not to reassess this malignancy in the present study, but to concentrate on non-neoplastic, in particular portal hypertension-related complications. However, when analyses were repeated while censoring patients diagnosed with HCC during follow-up, the cumulative incidence of major non-neoplastic complications was very comparable. Furthermore, while this is a multi-center, international study, not all ethnicities are well-

represented in our cohort. Interestingly, preliminary results of a Japanese study appear to confirm most of our results, particularly with regard to risk factors and prognosis[53]. Lastly, due to the nature of this study we cannot rule out that a selection bias might exist. Hence, since some relatively specialized centers are included in the cohort, incidence of major hepatic complications might be even lower in a general population of patients with PBC.

In conclusion, this large, international cohort study shows that in UDCA-treated PBC, major hepatic complications are not rare and develop in up to 15% of patients within 15 years of follow up. On the other hand, our data indicate that the incidence of these complications is decreasing over time, and that nowadays the large majority of patients treated with UDCA will remain free of serious complications such as ascites or variceal bleeding, and that subsequent survival has improved. Biochemical non-response to UDCA and high APRI are important risk factors for major hepatic complications.

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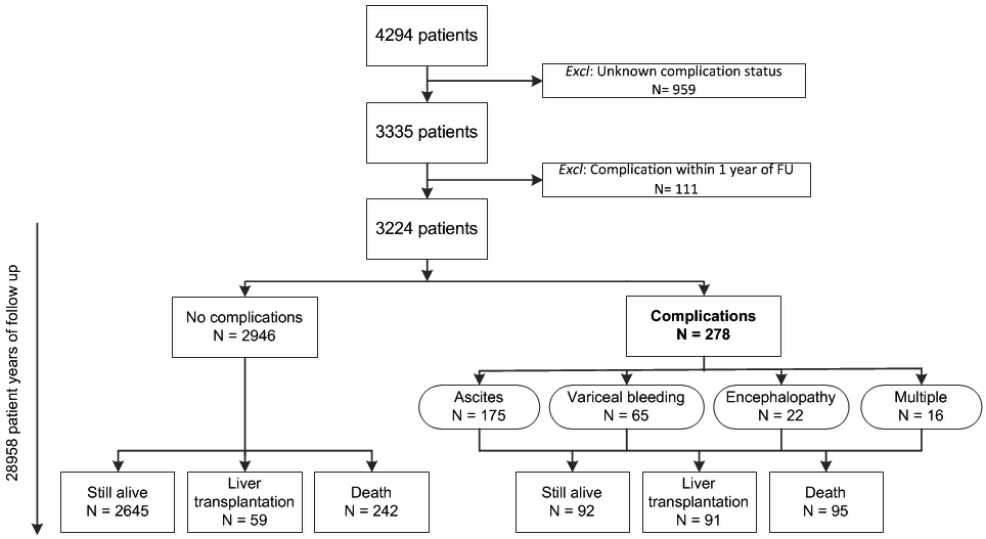
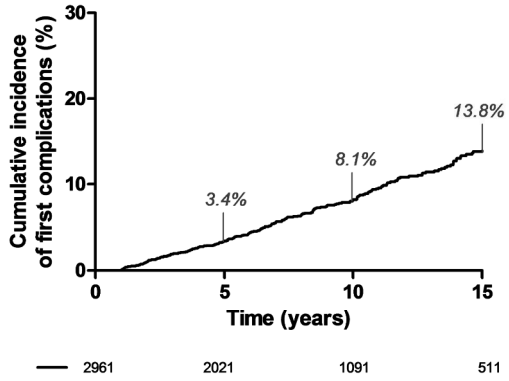
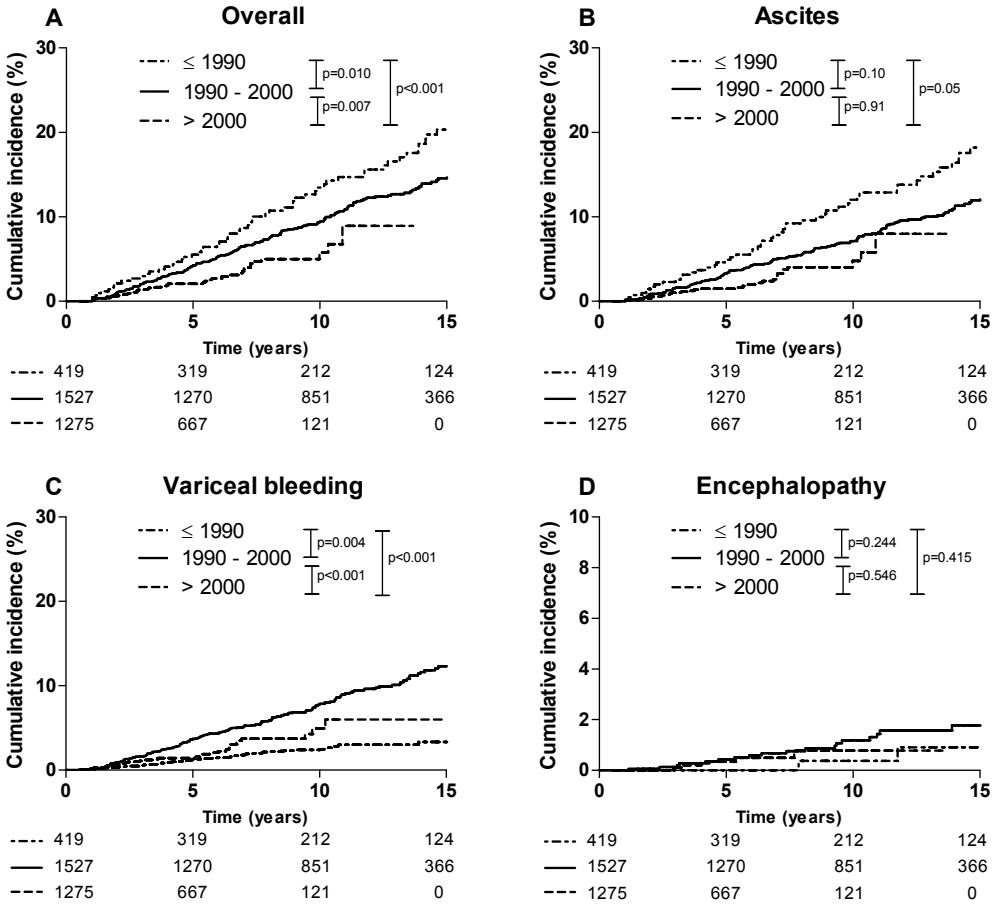
SUPPLEMENTARY DATA



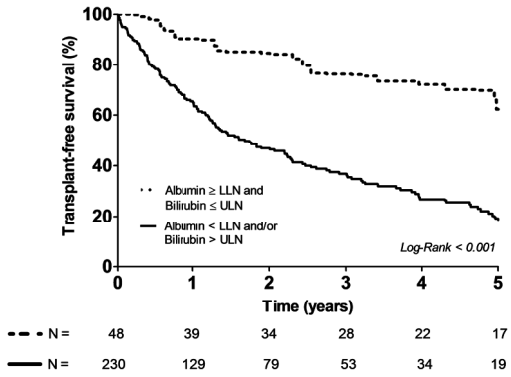
Supplementary Figure 1 Flowchart of study population. The initial study population consisted of 3335 patients, of whom 111 patients were excluded. The population used for analyses consists of 3224 patients.



Supplementary Figure 2 Cumulative incidence of major first hepatic complications in study cohort where all patients with a diagnosis of HCC before or at the same time of occurrence of major complications were censored at time of HCC diagnosis and patients with an unknown HCC status were excluded from analysis.



Supplementary Figure 3 A) Cumulative incidence of all first complications combined, stratified according the time period of patient inclusion; B) Cumulative incidence of ascites as first complication; C) Cumulative incidence of variceal bleeding as first complication; D) Cumulative incidence of encephalopathy as first complication. Please note that the sum of the incidences of the separate major complications (B, C and D) in the different time periods do not equal the totals (A) because of the existing competing risk of these endpoints.



Supplementary Figure 4. Transplantation-free survival following occurrence of hepatic complications according to serum albumin and total bilirubin levels.
Abbreviations: LLN, lower limit of normal; ULN, upper limit of normal.

Supplementary Table S1. Center-specific follow-up of the study population

		Follow-up (years)		
	N	**Range**	**Median (IQR)**	**Range**
The Netherlands (nationwide cohort)	738	1973 - 2012	8.9	0.6 – 24.2
Canada (Toronto)	462	1974 - 2011	7.7	0.9 – 34.3
Italy (Milan, 2 centers)	341	1968 - 2012	9.3	0.6 – 25.8
USA (Texas)	320	1977 - 2012	8.9	0.8 – 23.7
France (Paris)	303	1977 - 2011	6.2	0.6 – 22.5
Italy (Padua)	248	1976 - 2012	7.7	0.5 – 23.8
Spain (Barcelona)	244	1981 - 2012	12.7	0.6 – 23.8
Belgium (Leuven)	126	1974 - 2012	7.0	0.7 – 20.2
USA (Rochester)	117	1975 - 2012	3.0	0.6 – 17.3
Germany (Jena)	107	1979 - 2015	6.0	0.7 – 22.7
Greece (Larissa)	96	1991 - 2015	8.8	0.5 – 23.1
United Kingdom (London)	41	1983 - 2012	8.8	0.5 – 19.3
Canada (Edmonton)	31	1991 - 2012	6.5	1.8 – 18.4
USA *(Seattle)*	30	1995 - 2012	2.9	0.7 – 17.4
Gent	20	1996 - 2015	7.7	0.6 – 19.7

Abbreviations: IQR, interquartile range.

Supplementary Table S2. Baseline cohort characteristics in different time periods

	≤ 1990	1990 - 2000	> 2000
	N = 425	N = 1541	N = 1258
Age, years[a]	50.9 (11.2)	54.5 (12.4)	54.5 (12.4)
Female, n (%)	358 (90.6)	1405 (91.2)	1132 (90.0)
AMA+, n (%)	382 (90.0)	1398 (90.7)	1134 (90.1)
Year of diagnosis[b]	1986 (1983-1988)	1994 (1991-1997)	2005 (2002-2008)
Year of enrolment[b,c]	1989 (1988-1990)	1995 (1993-1998)	2005 (2002-2008)
Follow up, years[b]	10.9 (5.7-16.9)	11.3 (7.0-15.0)	5.3 (3.1-8.1)
Interval diagnosis - UDCA > 2 yrs, n (%)	242 (56.9)	469 (30.4)	118 (9.4)
Histological disease stage, n (%)[d]			
Stage I	60 (27.8)	335 (32.2)	335 (47.5)
Stage II	70 (32.4)	363 (34.9)	217 (30.8)
Stage III	46 (21.3)	158 (15.2)	110 (15.6)
Stage IV	40 (18.5)	185 (17.8)	43 (6.1)
Biochemical disease stage[e], n (%)			
Bilirubin≤ULN and albumin≥LLN	195 (63.3)	594 (75.8)	450 (74.9)
Bilirubin>ULN or albumin<LLN	99 (32.1)	159 (20.3)	123 (20.5)
Bilirubin>ULN and albumin<LLN	14 (4.5)	31 (3.8)	28 (4.7)
Serum total bilirubin ×ULN[b]	0.60 (0.43-0.93)	0.63 (0.47-0.90)	0.68 (0.47-1.1)
Serum ALP ×ULN[b]	2.17 (1.36-3.79)	2.25 (1.43-3.91)	2.02 (1.27-3.60)
Serum AST ×ULN[b]	1.50 (1.00-2.29)	1.50 (1.00-2.27)	1.38 (0.89-2.17)
Serum ALT ×ULN[b]	1.70 (1.05-2.72)	1.75 (1.10-2.75)	1.58 (0.96-2.53)
Serum albumin ×LLN[a]	1.16 (0.15)	1.17 (1.08-1.26)	1.14 (1.05-1.23)
Serum platelet count $\times 10^3/mm^3$[a]	254 (88)	243 (195-294)	237 (174-291)
APRI[f]	0.59 (0.37-0.93)	0.63 (0.40-1.00)	0.58 (0.33-1.05)

≤ 1990: Data on histological disease stage was missing for n=209 (49.2%), biochemical disease stage was missing for n=117 (27.5%,) total bilirubin was missing for n=90 (21.2%), ALP was missing for n=109 (25.6%), AST was missing for n=118 (27.8%), ALT was missing for n=118 (27.8%), albumin was missing for n=107 (25.2%), platelet count was missing for n=209 (49.2%).
1990 - 2000: Data on histological disease stage was missing for n=480 (31.1%), biochemical disease stage was missing for n=757 (49.1%,) total bilirubin was missing for n=511 (33.2%), ALP was missing for n=540 (35.0%), AST was missing for n=588 (38.1%), ALT was missing for n=596 (38.7%), albumin was missing for n=724 (47.0%), platelet count was missing for n=700 (45.4%).
> 2000: Data on histological disease stage was missing for n=553 (44.0%), biochemical disease stage was missing for n=657 (52.4%,) total bilirubin was missing for n=430 (34.2%), ALP was missing for n=365 (29.0%), AST was missing for n=364 (28.9%), ALT was missing for n=394 (31.3%), albumin was missing for n=635 (50.5%), platelet count was missing for n=608 (48.3%).
[a] Data is expressed as mean and standard deviation;
[b] Data is expressed as median and interquartile range;
[c] Equals the year of start of treatment with ursodeoxycholic acid;
Histological stage according to Ludwig and Scheuer's classification[27];
[e] Biochemical disease stage was classified according to the Rotterdam criteria[28];
[f] AST/platelets Ratio Index[16, 52];

Supplementary Table S3. *Factors associated with major hepatic complications after 12 months of UDCA therapy - a sensitivity analyses on complete cases*

	Univariate analysis			Multivariable analysis		
	HR	**95% CI**	**p**	**HR**	**95% CI**	**p**
Age *(per 10 years)*	1.10	0.98 - 1.23	0.103			
Age < 40 years	0.73	0.49 - 1.07	0.113			
Male sex	1.58	1.10 - 2.26	0.013			
AMA negative	1.35	0.84 - 2.17	0.209			
Year of diagnosis *(per 10)*	0.55	0.45 - 0.66	<0.001	0.85	0.63 - 1.14	0.278
Interval diagnosis - UDCA > 2 yrs[a]	1.92	1.49 - 2.48	<0.001			
Advanced disease[b]	5.23	3.97 - 7.07	<0.001			
Total bilirubin ×ULN[c]	3.85	3.22 - 4.59	<0.001			
ALP ×ULN[c]	2.43	1.97 - 2.99	<0.001			
AST ×ULN[c]	3.36	2.77 - 4.08	<0.001			
ALT ×ULN[c]	2.08	1.72 - 2.51	<0.001			
Albumin ×LLN	0.01	0.004 - 0.0.	<0.001			
Platelets *(per 50*x10^3/mm^3*)*	0.50	0.39 - 0.65	<0.001			
AST/ALT ratio[c]	1.55	1.28 - 2.41	0.003			
APRI[d], *continuous*[c]	2.22	1.56 - 3.15	<0.001			
APRI> 0.54	11.6	7.10 - 19.0	<0.001	8.88	5.25 - 15.0	<0.001
UK-PBC score *(per 20)*[e]	1.53	1.40 - 1.63	<0.001			
UK-PBC score ≤ median[e]	2.76	2.14 - 3.69	<0.001			
GLOBE-score, *continuous*	2.71	1.77 - 4.16	<0.001			
Biochemical non-response[f]	4.96	3.56 - 6.90	<0.001	3.40	2.19 - 5.26	<0.001

Total bilirubin was missing for n=703 (21.8%), ALP was missing for n=647 (20.6%), AST was missing for n=751 (23.3%), ALT was missing for n=722 (22.4%), albumin was missing for n=1365 (42.3%), platelet count was missing for n=1423 (44.1%).

[a] Years of untreated PBC diagnosis before starting UDCA therapy;

[b] Abnormal serum albumin and/or bilirubin[28];

[c] These variables were transformed with natural logarithm;

[d] AST/Platelets Ratio Index[16, 54];

[e] The median UK-PBC score in this study cohort was -103.3[31];

[f] Based on (age-dependent) GLOBE score thresholds[12];

Abbreviations: HR, hazard ratio; CI, confidence interval; AMA, anti-mitochondrial antibodies; ULN, upper limit of normal; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LLN, lower limit of normal; APRI, AST/Platelets Ratio Index.

Supplementary Table S4. Covariates associated with reduced transplant-free survival at the moment of hepatic complications.

	Univariate analysis				Multivariable analysis			
	HR	**95% CI**		**p**	**HR**	**95% CI**		**p**
Male sex	1.32	0.83	2.08	0.240				
AMA positive	1.01	0.62	1.64	0.979				
Age (per 10 years)	1.13	0.98	1.30	0.106				
Calendar year (per 10 years)	0.76	0.69	0.83	0.057	0.72	0.54	0.95	0.022
Total bilirubin	2.41	1.99	2.93	<0.001				
ALP	1.33	1.05	1.69	0.019				
AST	1.98	1.55	2.54	<0.001				
ALT[a]	1.23	0.98	1.53	0.076				
AST/ALT ratio	2.50	1.72	3.64	<0.001				
APRI	1.33	1.10	1.61	0.003				
Albumin	0.65	0.52	0.82	<0.001				
Platelets (per 50x10³/mm³ decline)	1.06	0.97	1.15	0.238				
Total bilirubin > ULN	2.86	2.00	4.09	<0.001	2.75	1.91	3.96	<0.001
ALP > 2x ULN	1.46	1.09	1.95	0.012				
ALT > 2x ULN	1.10	0.79	1.53	0.574				
AST > 2x ULN	1.73	1.28	2.32	<0.001				
Albumin < LLN	2.24	1.63	3.08	<0.001	2.45	1.58	3.83	<0.001
Platelets < 100 (x10³/mm³)	1.01	0.74	1.36	0.997				
APRI > 2x ULN	1.81	1.34	2.44	<0.001				
AST/ALT ratio > ULN	2.20	1.41	3.43	0.001				

a. These variables were transformed with natural logarithm.
Abbreviations: AMA, anti-mitochondrial antibodies; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, AST to Platelets Ratio Index; ULN, upper limit of normal; LLN, lower limit of normal

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CHAPTER 4
URSODEOXYCHOLIC ACID TREATMENT AND LIVER TRANSPLANTATION-FREE SURVIVAL IN PATIENTS WITH PRIMARY BILIARY CHOLANGITIS

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ABSTRACT

Background and aims The clinical efficacy of ursodeoxycholic acid (UDCA) in primary biliary cholangitis (PBC) remains subject to debate as definitive randomized controlled trials are lacking. We aimed to determine whether UDCA prolongs transplantation (LT) free survival in PBC.

Methods This international cohort study included patients from the Global PBC Study Group database, originating from 8 countries in Europe and North America. Both UDCA-treated and untreated patients were included. LT and death were assessed as a combined endpoint through Cox regression analyses, with inverse probability of treatment-weighing (IPTW).

Results In the 3902 patients included, the mean (SD) age was 54.3 (11.9) years, 3552 patients (94.0%) were female, 3529 patients (90.4%) were treated with UDCA and 373 patients (9.6%) were not treated. The median (IQR) follow-up was 7.8 (4.1-12.1) years. In total, 721 UDCA-treated patients and 145 untreated patients died or underwent LT. After IPTW, the 10-year cumulative LT-free survival was 79.7% (95%CI 78.1-81.2) among UDCA-treated patients and 60.7% (95%CI 58.2-63.4) among untreated patients (P<0.001). UDCA was associated with a statistically significant reduced risk of LT or death (Hazard Ratio [HR] 0.46, 95%CI 0.40-0.52, P<0.001). The HR remained statistically significant in all stages of disease. Patients classified as inadequate biochemical responders after one year of UDCA had a lower risk of LT or death than patients who were not treated (adjusted HR 0.56, 95%CI 0.45-0.69, *P*<0.001).

Conclusion The use of UDCA improves LT-free survival among patients with PBC, regardless of the disease stage and the observed biochemical response. These findings support UDCA as the current universal standard of care in PBC.

INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic and usually slowly progressive liver disease with autoimmune features, histologically characterized by destruction of the small intrahepatic bile ducts[1, 2]. The disease is primarily diagnosed based on an otherwise unexplained chronic elevation of serum alkaline phosphatase levels and the presence of anti-mitochondrial antibodies. Early identification of individuals with PBC is clinically challenging as symptoms are frequently absent. Identifying and managing patients with PBC is important, however, as the disease may silently progress towards cirrhosis and the survival of affected patients is substantially impaired[3].

UDCA is a choleretic and hydrophilic endogenous bile acid that is considered a safe and well-tolerated drug[4-6]. Based on the cumulative experience obtained with this drug over the past decades, UDCA is recommended as the standard treatment for PBC[4, 6]. Long-term cohort studies have suggested an association between UDCA and improved LT-free survival, but this was only based on the comparison of observed versus predicted LT-free survival according to the Mayo Risk Score, which estimates the prognosis when patients are left untreated[7-9]. Numerous randomized controlled trials (RCTs) have been performed as well, but all failed to show a difference in LT-free survival between placebo and UDCA treated groups[10-19]. As did other more extensive meta-analyses, the Cochrane hepatobiliary group recently concluded once again that there is no demonstrated benefit of UDCA on LT and/or mortality[17-20]. Such positioning statements, in absence of the results of definitive trials, have fueled the ongoing discussion about the therapeutic potential of UDCA[21-24]. This might explain the observation in a well-executed national PBC registry that, until recently, as much as 20% of patients remained untreated[25]. In another recent US-based cohort study the percentage of UDCA untreated patients was even as high as 30%[26]. However, the meta-analyses are based on inadequate RCTs that were limited by a small number of patients, insufficient dosages of UDCA, and short follow-up. Therefore, the evaluation of the clinical efficacy of UDCA in PBC should not be based on these RCTs alone. Nonetheless, there is an understandable reluctance to initiate new long-term, placebo-controlled RCTs in which many patients would be denied UDCA therapy, because of minimal safety concerns of UDCA and practical implications.

To support current practice, alternative study designs are thus needed to assess the potential benefit of treatment with UDCA in PBC. This would be relevant both to increase awareness for timely diagnosis and referral by physicians working in other fields, and to optimize future patient management by PBC-treating physicians. A contemporary causal inference method - used to emulate a randomized controlled trial in observational data - is inverse probability of treatment weighting (IPTW). The Global PBC Study Group cohort, which includes long-term follow-up data of both UDCA-treated and untreated patients, provides the opportunity to

apply this method. In our first publication, we substantiated alkaline phosphatase (ALP) and bilirubin as surrogate markers for clinical outcome in our cohort of 4845 patients with PBC[27]. In the second Gastroenterology publication, in which only the 4119 UDCA-treated patients were included, we developed the GLOBE score, a model that accurately predicts long-term outcome[28]. In the current study performed in this cohort, we aimed to assess the effect of UDCA therapy on LT-free survival. The second objective was to evaluate the difference in LT-free survival between patients who do not meet biochemical criteria for response after one year of UDCA therapy and patients who remained untreated.

PATIENTS AND METHODS

Study population and design

Patients were derived from the Global PBC Study Group database. This study group is an international collaboration between 15 liver units across 8 countries in Europe and Northern America. The database contains individual patient data from long-term follow-up cohorts. Both UDCA-treated and untreated patients with an established diagnosis of PBC in accordance with internationally accepted guidelines[4-6] were eligible for inclusion in this study. In order to be eligible, we required the absence of confirmed chronic hepatitis B virus or chronic hepatitis C virus infection, Wilson' disease, alpha-1 antitrypsin deficiency, hereditary haemochromatosis, alcoholic liver disease, or overt overlapping features with autoimmune hepatitis. We then excluded patients from analysis in case of insufficient follow-up data (<6 months follow-up or <2 visits recorded, also in case of an endpoint within 6 months of follow-up), and when dates of starting treatment or clinical events were unknown. The centers involved in the current study followed their patients according to international guidelines, which includes a clinical assessment at least annually in absence of cirrhosis and at least 6-monthly in case of advanced disease[4, 6]. Cirrhosis was defined histologically as described by Ludwig[29, 30]. Methodology of data collection has previously been described in further detail[27]. For the current study 3902 of the 4845 patients included in the original cohort were assessed [27]. Eighty-six patients were excluded because it was not known whether these patients were or were not treated with UDCA. In addition, one center is currently withdrawn from the Global PBC Study Group. This study was conducted in accordance with 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 their local regulations.

Statistical analysis

The primary endpoint was defined as a composite endpoint of LT and all-cause mortality. As a secondary endpoint liver-related morbidity was assessed, defined by the composite of specific liver-related events (ascites, variceal bleeding, hepatic encephalopathy,

hepatocellular carcinoma) or a clinical condition resulting in the need of LT, whichever came first. Baseline was defined as the first center visit for untreated patients. For UDCA-treated patients, the start date of UDCA-therapy was considered baseline. In PBC, treatment is lifelong and is initiated prompt after diagnosis (in this study: median 2.9 months, interquartile range (IQR) 0-29 months). Because PBC is a relatively slowly progressing disease, this treatment is commonly initiated long before endpoints occur. Therefore, UDCA was not analyzed as a time-dependent covariate. When no events occurred during follow-up, patients were censored at time of their last center visit.

Because treatment was not assigned randomly in our study population and baseline variables could influence both the chance of mortality or LT, as well as the chance of receiving treatment (i.e. time-dependent confounding), inverse probability treatment weighting (IPTW) was used to estimate the outcomes[31]. Weights were assigned to each individual patient. In order to create the weights, a logistic regression model was created that included independently significant baseline characteristics and laboratory parameters (age, gender, calendar year of diagnosis, total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count, and albumin), in which UDCA therapy was the dependent variable. The model's predictive values were saved. Weights were subsequently estimated as per (1/predicted value) for UDCA-treated patients and (1/(1-predicted value)) for untreated patients. Subsequently, the weights were stabilized[32]. Balance assessment was then performed by evaluating differences between the treated and untreated patient groups after weighting[33] (**Supplementary Figure S1**). The hazard ratio (HR) of UDCA therapy was calculated by Cox proportional hazard regression analyses. In observational data, immortal time (person-time accumulated between date of diagnosis and date of treatment initiation) bias can potentially lead to overestimation of treatment effect[34]. For this reason, a sensitivity analyses including only patients diagnosed in or after 1990, when UDCA was universally available and usually initiated promptly after diagnosis, was performed.

The association between UDCA and LT-free survival was also explored in patients classified as non-responder[35] or inadequate responder[4] to UDCA. The recently developed GLOBE score, calculated after 12 months, was used as primary measure of response to UDCA[28]. We applied the score's age-specific thresholds, that categorize patients into either having an estimated prognosis similar to an age and sex matched general population, or an impaired estimated survival. Sensitivity analyses using other response criteria were performed. To ensure comparable follow-up time, we adjusted the starting point of follow-up of untreated patients according to the moment of assessing biochemical response. The Cox proportional hazard regression models used for these analyses were adjusted for patient demographics and biochemistry to correct baseline differences between the groups classified as responder, non-responder, and untreated population, respectively. Giving the power of our dataset, we

constructed a conservative model with extensive adjustment for baseline factors in order to estimate the association between UDCA and LT-free survival, adjusting for sex, age, year of diagnosis, serum total bilirubin, platelet count, albumin, ALP, AST, and ALT.

Interactions between UDCA and patient characteristics and baseline laboratory values were explored for significance. Where indicated, continuous variables were transformed to their natural logarithm to correct for non-linearity. To correct for missing laboratory values, ten databases generated by means of multiple imputations (SAS Proc MI, MCMC method), were used for analyses[36, 37].We assumed missing data occurred at random. Rubin's rules were used for estimation of the parameters and the standard error[38, 39]. The imputation model variables included both those potentially predicting outcome and outcomes themselves. The (continuous) biochemical values were imputed at baseline, after one year, and after two years of follow-up. The biochemical values included for imputation were: ALP, AST, ALT, total bilirubin, albumin, and platelet count. In case of non-normality, the natural logarithm of these variables was used. No categorical or binary variables were imputed.

All statistical tests were two-sided, and a P-value <0.05 was considered to be statistically significant. The significance level for interactions was set at p=0.01 to correct for multiple testing. Statistical analyses were performed in SPSS Statistics V.21.0 (Armonk, NY: IBM Corp.) and SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Study population

In total, 3902 patients with PBC were included. At baseline, the mean (standard deviation) age was 54.3 (11.9) and, the vast majority of patients was female (n= 3552, 91.0%). Three thousand five hundred and twenty-nine patients (90.4%) were treated with UDCA and 373 patients (9.6%) were not treated with UDCA. **Table 1** shows the baseline characteristics according to the treatment with UDCA. Patients treated with UDCA were younger and had higher circulating serum liver tests, while in the subgroup of patients with available baseline histology, on average, patients not treated with UDCA had more advanced stages of disease. Although statistically significant, these numerical differences were small. Coinciding with the gradual more widespread introduction of UDCA treatment since the early nineties of the last century, the median year of diagnosis was earlier in untreated patients (1992, interquartile range [IQR] 1982-2000) as compared to UDCA-treated patients (1997, IQR 1990-2003). Balance assessment showed no remaining statistically significant differences regarding baseline patient characteristics between the untreated and the UDCA-treated population after adjustment with IPTW (**Supplementary Figure S1**).

Liver Transplantation-free survival according to UDCA therapy

During a median follow-up duration of 7.8 (IQR 4.1-12.1) years 299 patients underwent LT and 567 patients died. LT or death (as a combined endpoint) was reached by 721 UDCA-treated patients and 145 untreated patients. The incidence rate of LT or death was 23.21 per 1000 person-years (95% confidence interval [CI] 21.52-24.91) in patients treated with UDCA and 58.81 per 1000 person-years (95% CI 49.24-68.38) in patients not treated with UDCA (p<0.001). After IPTW adjustment, the 5-year cumulative LT-free survival was 90.8% (95% CI 90.0-91.7) among UDCA-treated patients and 81.0% (95% CI 79.3 – 82.7) among untreated patients (**Figure 1)**. At 10 years of follow-up, the cumulative LT-free survival rates were 79.7% (95% CI 78.1-81.2) and 60.7% (95% CI 58.2-63.4), respectively (**Table 2**). Weights-adjusted Cox proportional hazard regression analyses showed that UDCA therapy was associated with a statistically significant reduction in the hazard of LT or death (Hazard Ratio [HR] 0.46 (95%CI 0.40-0.52, p<0.001). 1958 of 3902 patients (50%) could be included in analyses regarding the dosage of UDCA. The association between UDCA therapy and improved LT-free survival remained statistically significant among those treated with <13 mg/kg (n=914) of UDCA (HR 0.50, 95%CI 0.43-0.57, p<0.001), but was stronger for patients treated with ≥13 mg/kg of UDCA (n=671) (HR 0.29, 95%CI 0.21-0.39, p<0.001). In the study cohort of 3902 patients, data on liver-related morbidity was available for 2982 (76.4%) patients, of whom 266 were untreated, and 2716 were UDCA-treated. In total, 381 events were found. After 10 years of follow-up, the weights-adjusted cumulative incidence of liver-related morbidity

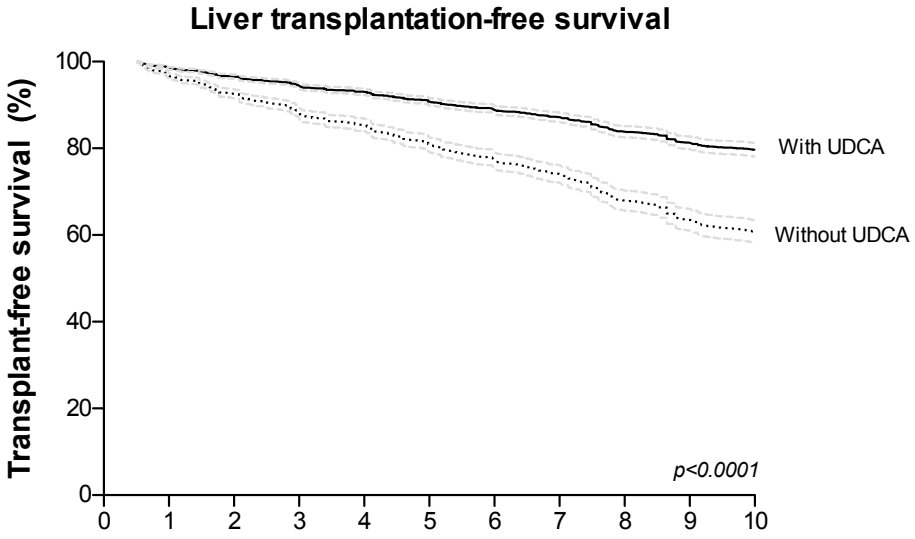

Figure 1. Transplant-free survival according to UDCA treatment. The solid line represents the weights-adjusted survival of UDCA-treated patients (n=3529), the dotted line reflects the weights-adjusted survival of untreated patients (n=373) (p<.001). The 95% confidence intervals are reflected by the grey lines. The survival figure was constructed using an IPTW-adjusted Cox proportional hazard model.
Abbreviations: UDCA, ursodeoxycholic acid; IPTW, inverse probability of treatment weighting.

Table 1. Baseline characteristics

	Overall N = 3902	UDCA-treated N = 3529	Untreated N = 373	P value
Age at diagnosis, years	52.3 (11.9)	52.1 (11.7)	54.1 (13.4)	<0.001
Female, n (%)	3552/3902 (91.0)	3209/3529 (90.9)	343/373 (92.0)	0.510
AMA positive, n (%)	3507/3862 (90.8)	3175/3491 (90.9)	332/371 (89.5)	0.418
Year of diagnosis	1996 (1990-2003)	1997 (1990-2003)	1992 (1982-2000)	<0.001
Histological disease stage, n (%)				<0.001
Stage I	784/2173 (36.1)	739/2076 (35.6)	45/97 (46.4)	
Stage II	671/2173 (30.9)	657/2076 (31.6)	14/97 (14.4)	
Stage III	365/2173 (16.8)	351/2076 (16.9)	14/97 (14.4)	
Stage IV	353/2173 (16.2)	329/2076 (15.8)	24/97 (24.7)	
Serum bilirubin (ULN)	0.63 (0.44-1.00)	0.62 (0.44-1.00)	0.65 (0.43-1.38)	0.081
Serum ALP (ULN)	2.29 (1.41-3.95)	2.32 (1.46-4.00)	1.94 (1.11-3.51)	<0.001
Serum AST (ULN)	1.53 (1.03-2.31)	1.56 (1.05-2.34)	1.25 (0.75-2.00)	<0.001
Serum ALT (ULN)	1.68 (1.05-2.63)	1.71 (1.09-2.68)	1.20 (0.75-1.83)	<0.001
Serum albumin (LLN)	1.15 (1.06-1.25)	1.15 (1.06-1.25)	1.15 (1.03-1.26)	0.840
Platelet count ($x10^3/mm^3$)	245 (190-300)	248 (195-303)	217 (146-271)	<0.001
Biochemical disease stage, n (%)				<0.001
Early	1576/2296 (68.6)	1376/1980 (69.5)	200/316 (63.3)	
Advanced	559/2296 (24.3)	484/1980 (24.4)	75/316 (23.7)	
Severe	161/2296 (7.0)	120/1980 (6.1)	41/316 (13.0)	

Abbreviations: UDCA, ursodeoxycholic acid; AMA, anti-mitochondrial antibodies; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

was 27.6% (95%CI 24.4-30.6) among the patients without UDCA and 13.5% (95%CI 11.8-15.1) among those with UDCA (p<0.001). In weights-adjusted Cox regression analyses UDCA therapy was associated with a statistically significant reduction in the hazard of liver-related morbidity (HR 0.45, 95%CI 0.36-0.55, p<0.001). In a sensitivity analysis in the subcohort diagnosed ≥1990, in which the median interval between diagnosis and initiation of UDCA treatment was 0.096 years (IQR 0.000-0.586), we found a similar association between UDCA and LT-free survival (HR 0.38, 95%CI 0.32-0.46).

Association between UDCA and Liver Transplantation or death in subgroups

In order to assess the stability of the association between UDCA therapy and improved LT-free survival, the IPTW-adjusted survival analyses were stratified according to various categorized baseline characteristics. The association between UDCA and improved LT-free survival was statistically significant in both males and females, younger and older patients, patients with early disease and patients with more advanced disease, as well as patients

Table 2. Clinical endpoints, incidence rates and liver transplantation-free survival according to the use of UDCA.

	With UDCA	Without UDCA	P Value
No. of clinical endpoints[a]	721	145	
Incidence rate *per 1000 person-years*[b]	23.2 (21.5-24.9)	58.8 (49.2-63.4)	<.001
5-year cumulative LT-free survival (%)[b,c]	90.8 (90.0-91.7)	81.0 (79.3-82.7)	<.001
10-year cumulative LT-free survival (%)[b,c]	79.7 (78.1-81.2)	60.7 (58.2-63.4)	<.001

P value were assessed using Cox proportional hazard analyses and the Chi2 contingency table.
[a] Liver transplantation or death
[b] Reported with 95% confidence interval
[c] Adjusted using inverse probabily treatment weighting
Abbreviations: No, number; UDCA, ursodeoxycholic acid; LT, liver transplantation.

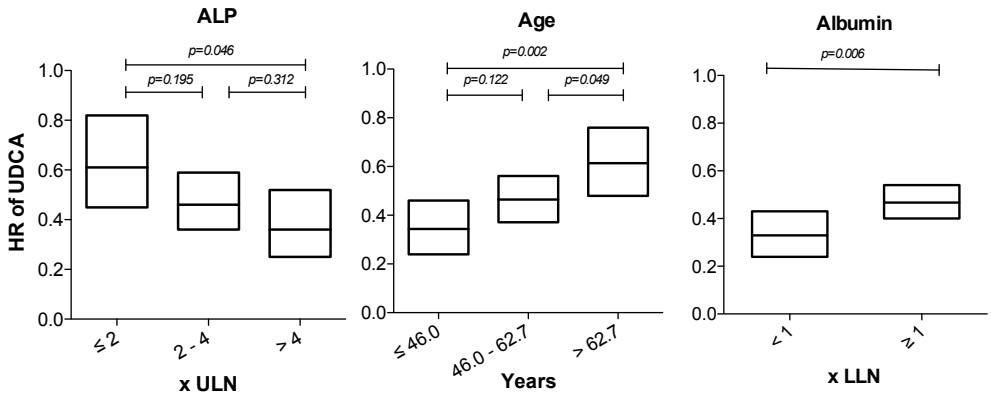

Fig. 2. Stratified association between UDCA therapy and transplant-free survival according to baseline serum ALP and albumin levels and age groups. Assessed using an IPTW-adjusted Cox proportional hazard model. A) adjusted HR of UDCA according to baseline alkaline phosphatase (x ULN); B) adjusted HR of UDCA according to baseline age, showing the youngest quartile, the middle 50%, and the oldest quartile; C) adjusted HR of UDCA according to baseline albumin (for which a definite explanation). The bars represent the weights-adjusted hazard ratios of UDCA and their 95% confidence intervals.
Abbreviations: ALP, alkaline phosphatase; IPTW, inverse probability of treatment weighting; HR, hazard ratio; UDCA, ursodeoxycholic acid; ULN, upper limit of normal; LLN, lower limit of normal.

with a favorable and an unfavorable biochemical profile (**Table 3**, **Supplementary Figure S3**). The HRs of UDCA with respect to LT or death were statistically significant among all subgroups of patients (**Table 3**). The estimated HRs differed according to baseline age, ALP, and albumin (**Figure 2**). The interaction terms with UDCA were statistically significant for age and albumin.

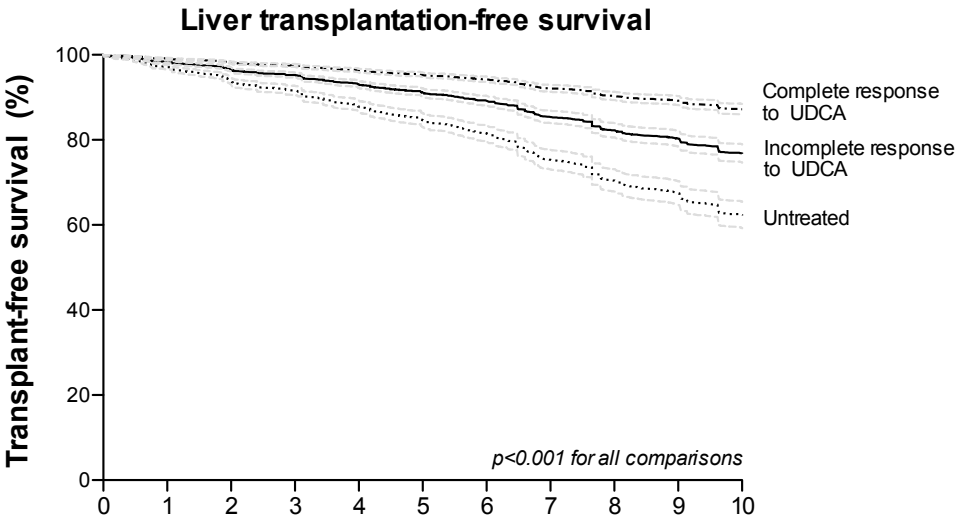

Fig. 3. Transplant-free survival stratified after 12 months of follow-up for treatment response versus no UDCA treatment. Survival figures were constructed using an IPTW-adjusted Cox proportional hazard model. The grey line represents the weights-adjusted survival of untreated patients (n=373), black solid line reflects the adjusted survival of patients classified as incomplete responder (n=733) according to the GLOBE score[28] and the dotted line reflects the adjusted survival of patients classified as complete responder (n=2700) according to the GLOBE score. All curves were adjusted for sex, age, year of diagnosis, bilirubin, albumin, platelet count, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase. The 95% confidences interval is reflected by the light grey dotted lines.
Abbreviations: UDCA, ursodeoxycholic acid; IPTW, inverse probability of treatment weighting.

Liver Transplant-free survival according to biochemical response to UDCA

Among the 3529 UDCA-treated patients, 3433 had a follow up of at least 12 months. Of these 3433 patients, 733 patients (21.4%) were classified as inadequate responders according to the GLOBE score 1 year after the start of UDCA therapy. After these initial 12 months, the adjusted cumulative 5-year LT-free survival was 95.3% (95% CI 94.8-95.9) in UDCA responders and 91.2% (95% CI 90.2-95.9) in patients with an inadequate response to UDCA, as opposed to 84.7% (95% CI 83.1-86.4) in the untreated patients (**Figure 3**). Multivariate Cox regression analysis showed that patients with inadequate response to UDCA had a statistically significant lower LT or death rate as compared to untreated patients (adjusted HR 0.56, 95%CI 0.45-0.69, p<0.001), but the favorable LT-free survival as opposed to those without therapy was stronger in UDCA responders (adjusted HR 0.25, 95%CI 0.20-0.30, p<0.001). These results were similar when response was assessed after 24 months (adjusted HR 0.62, 95%CI 0.52-0.74, p<0.001 and adjusted HR 0.27, 95%CI 0.22-0.33, p<0.001) and when applying other response criteria (Paris I, Paris II, Rotterdam, Toronto or Barcelona) (**Supplementary Table S1**)

Table 3. Stratified association between UDCA therapy and liver transplant-free survival

Characteristic		N	HR of UDCA[a]	95% CI	p-value HR UDCA	p-value Interaction
Sex						0.789
	Male	350	0.52	0.35-0.77	0.0011	
	Female	3552	0.44	0.38-0.52	<0.0001	
Age, *years*						
Reference	≤46.0	974	0.33	0.24-0.46	<0.0001	
	46.0-62.7	1948	0.46	0.37-0.56	<0.0001	0.122
	>62.7	979	0.60	0.48-0.76	<0.0001	0.002
Cirrhosis[b]						0.312
	No	1820	0.32	0.24-0.42	<0.0001	
	Yes	353	0.31	0.24-0.40	<0.0001	
Biochemical disease stage[c]						
	Early	2649	0.37	0.30-0.47	<0.0001	
	Intermediate	985	0.32	0.25-0.40	<0.0001	0.196
	Advanced	268	0.50	0.37-0.70	<0.0001	0.271
ALP						
Reference	≤ 2x ULN	1679	0.61	0.45-0.82	0.0014	
	2-4x ULN	1285	0.46	0.36-0.59	<0.0001	0.195
	> 4x ULN	938	0.36	0.25-0.52	<0.0001	0.046
Bilirubin						0.334
	≤ ULN	2930	0.39	0.32-0.48	<0.0001	
	> ULN	972	0.40	0.33-0.48	<0.0001	
Albumin						0.006
	< LLN	549	0.32	0.24-0.43	<0.0001	
	≥ LLN	3353	0.46	0.40-0.54	<0.0001	
Platelet count						0.951
	< $150x10^9$	531	0.48	0.35-0.65	<0.0001	
	≥ $150x10^9$	3371	0.44	0.37-0.52	<0.0001	

P-values were assessed using IPTW-adjusted Cox proportional hazard models.

[a] The Hazard Ratios were adjusted for the weights;

[b] Baseline histological data was available for 2173 patients;

[c] Biochemical disease stage according to Rotterdam criteria[8].

Abbreviations: HR, hazard ratio; CI, confidence interval; UDCA, ursodeoxycholic acid; ALP, alkaline phosphatase; ULN, upper limit of normal; LLN, lower limit of normal; IPTW, inverse probability of treatment weighting.

DISCUSSION

In this large, international follow-up study including both UDCA-treated and untreated patients, we report that UDCA therapy improves LT-free survival in PBC, with a dose-response relationship. Importantly, a statistically significant association between UDCA therapy and reduced all-cause mortality or LT was found in all stages of disease. These findings imply a strong recommendation for all patients with PBC to use UDCA. Even in UDCA-treated patients classified as inadequately responding to UDCA according to accepted criteria, an improved LT-free survival was found in comparison to untreated patients. This indicates that UDCA should not be stopped in these inadequate responders and that future therapeutic options for this patient group should initially be considered as add-on medication. Additionally, our results underline the importance of adequate dosing of UDCA of at least 13 mg/kg.

The 2.2 fold risk reduction associated with UDCA treatment that we report is more pronounced than in the previous (meta-)analyses that quantified the benefit of UDCA with relative risk reductions of approximately 1.5[40-42]. This may be explained by the longer follow-up and subsequent higher incidence of clinical endpoints in our study cohort, but also by the use of more adequate dosages of UDCA over time and the subsequent larger associated risk reduction. While most previous studies did not establish evidence for a clinical benefit of UDCA at all, one combined analysis of three of the available RCTs did report a significantly improved survival in patients with advanced disease[40]. Irrespective of disease severity, a clear understanding about the potential impact of UDCA is relevant for all patients, for patient counseling and therapeutic compliance, but also for cost justification. An important novelty of the current study is thus the encouraging demonstration of a statistically significant association between UDCA therapy and prolonged LT-free survival throughout all subgroups of PBC patients, including those with and without cirrhosis, and irrespective of biochemical disease stage or other baseline biochemistry. This finding opposes the widely held belief that UDCA may be particularly useful in early stage disease. Although the aforementioned combined analysis suggested a therapeutic benefit in advanced disease[40], a clear beneficial effect of UDCA in late stage PBC has often been considered doubtful or even unlikely[24, 43]. We did not identify any subgroup of patients without an improved LT-free survival associated with UDCA therapy, even when subgroups were further stratified into more extreme values of biochemistry and age (data not shown). These analyses were possible due to the large number of patients and long follow-up duration in this study. Prior studies, and especially prior RCTs, were lacking such power and this has indeed been the major criticism regarding the lacking evidence of clinical gain of UDCA therapy in PBC to date. Yet, these prior studies may have contributed to the fact that still not all patients with PBC are receiving UDCA treatment today, despite the recommendations in current international guidelines[4, 6]. A recent real-life American cohort study revealed that 30% of patients remained untreated[44],

and a similar percentage of untreated patients is reported in a yet unpublished German study which included patients diagnosed after 2015.

Our analyses showed that in younger PBC patients, there is a stronger LT-free survival benefit of UDCA than in older patients. In the elderly, survival is also driven by extrahepatic factors which are unlikely to be influenced by UDCA, attenuating the HR. We are lacking detailed data on cause of death for further clarification. This result might seem counterintuitive as previous studies showed that young PBC patients are less likely to meet the criteria for response to UDCA after one year of treatment, that are mainly based on liver blood tests after 12 months of treatment[9, 25, 45-50]. It should be realized, however, that the biochemistry of young patients is often worse than that of older patients (confirmed in our cohort, data not shown) and that achievement of crude dichotomous biochemical response criteria is related to the baseline level of these laboratory parameters[51]. The frequently applied response criteria evaluate neither absolute nor relative improvements within the individual. Patients with high levels of ALP are therefore likely to realize major improvements of their biochemistry, with considerable clinical benefit, while still being classified as non-responders or, at least, inadequate responders. Indeed, here we show that the relative risk reduction of LT or death associated with UDCA therapy is greater among those patients with higher baseline ALP.

Another finding of importance is that among patients classified as inadequate responder according to the different international response criteria, when adjusted for relevant baseline predictors of both biochemical response and long-term outcome, the risk of LT or death with UDCA treatment was still 1.8-fold lower as compared to patients that were left untreated. Nonetheless, this effect was more pronounced in patients classified as responder, who have been shown to have a survival comparable to the general population[28]. While response criteria are clearly well able to identify patients in need of second-line treatment, not meeting these criteria should not be interpreted as an absence of treatment effect. Denomination of either 'non-response' or 'inadequate response' to UDCA may therefore be inappropriate, as these terms do not capture the remaining therapeutic benefit in these patients. Incomplete response may be a more suitable alternative. While various second-line treatment options are currently emerging for PBC, our result stresses the importance not to withhold patients from UDCA therapy. UDCA has been extensively studied on long-term effects and a causal benefit of UDCA on survival seems likely, even more so because of the results of the IPTW analyses in our study, including the dose-response association. Furthermore, UDCA has proven to be very safe when adequately dosed and is inexpensive[4, 6, 52]. At present, the novel therapies for PBC should thus be primarily considered as add-on treatment. Further studies should assess whether monotherapy with these new drugs has the potential to result in a similar or superior clinical benefit.

Our study comprises both strengths and limitations. Although our study is not a RCT, we make use of a large real life cohort of both treated and untreated patients. This previously enabled the in-depth assessment of biochemical surrogate markers in PBC, which led to the development and validation of the GLOBE score[27, 28]. The novelty of the study we present here is that we assessed the association between UDCA and LT-free survival by applying IPTW estimates, so that the power of the entire cohort was preserved. IPTW is a causal inference method, developed to emulate RCTs in observational data[53]. The long-term RCT that would be required to ultimately prove that the relation between UDCA therapy and improved prognosis is causal would be both hugely difficult in terms of practicality, and would generally be considered as unethical by having to withhold patients from UDCA treatment for many years. While the limitations of existing randomized controlled trials were extensively discussed, the current study is not free from limitations either. Residual confounding can never be ruled out in our cohort study in which the reasons for non-treatment are also unknown. However, it would be misleading to refrain from causal language since it is clearly the aim of this study to contribute to the body of evidence for the therapeutic effect of UDCA[54]. Moreover, because of the favorable safety profile, there are no evident contra-indications for the use of UDCA. Thus, it is difficult to imagine which unmeasured and unevenly distributed patient characteristic would completely diminish the strong association between UDCA and a prolonged LT-free survival. Secondly, time-dependent bias such as immortal time could potentially have led to overestimation of the association between UDCA and LT-free survival. However, in our sensitivity analyses among patients included from 1990 onwards, in which the time between diagnosis and start of UDCA was generally very short, the HR was similar. In order to ensure sufficient power for subgroup analyses, we preserved the entire cohort for all primary analyses. Moreover, our overall estimate might be considered conservative as we found a stronger HR in patients receiving an adequate dose of UDCA (>13 mg/kg), which is the regular dosage used today. Thirdly, we were not able to analyze all-cause mortality as a solitary endpoint, because we lack follow-up data after an event of LT. However, today LT-free survival is considered clinically most relevant in PBC and is thus used as primary endpoint in recent studies and by regulating authorities. Furthermore, because of the nature of this study and the fact that liver biopsy is no longer required for the diagnosis of PBC, our histology data were incomplete. Data on fibrate therapy, which was recently shown to have a beneficial effect on surrogate end-points in PBC[55], is not available in our study. However, this is unlikely to have had a major influence on our results as only a minority of the more recent patients in our large cohort may have received off-label treatment with fibrates.

In conclusion, this large multi-center study indicates that UDCA therapy improves LT-free survival in all patients with PBC, both in those with early and advanced disease, as well as in patients not meeting accepted criteria for response to UDCA.

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SUPPLEMENTARY DATA

Supplementary Table S1. Adjusted HR of UDCA treatment for inadequate response versus no UDCA treatment and response to UDCA versus no UDCA treatment, according to the different international response criteria for response to UDCA treatment.

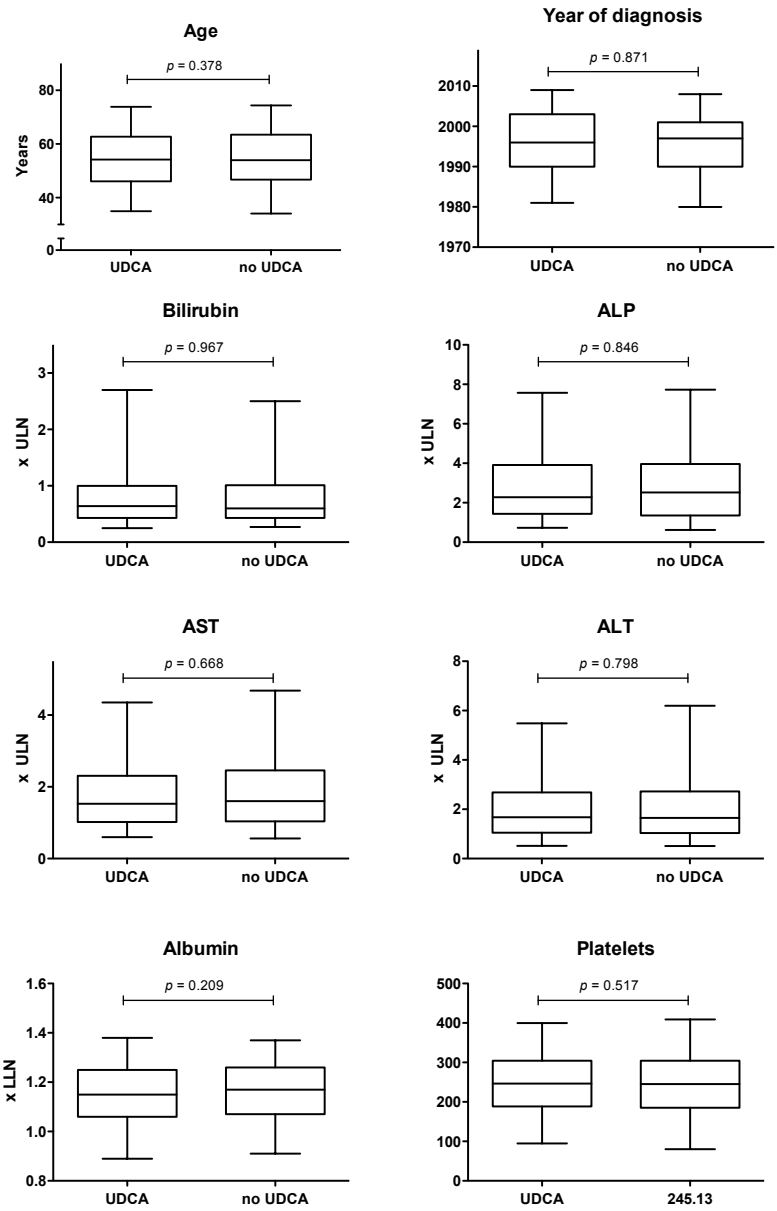
	Adjusted HR UDCA[a]	95% CI	P value
GLOBE			
Inadequate response vs. no UDCA	0·558	0·453-0·686	<·0001
Response vs. no UDCA	0·289	0·234-0·357	<·0001
Paris II			
Inadequate response vs. no UDCA	0·459	0·381-0·553	<·0001
Response vs. no UDCA	0·297	0·231-0·380	<·0001
Toronto			
Inadequate response vs. no UDCA	0·461	0·368-0·576	<·0001
Response vs. no UDCA	0·317	0·259-0·387	<·0001
Rotterdam			
Inadequate response vs. no UDCA	0·504	0·325-0·784	<·0001
Response vs. no UDCA	0·389	0·329-0·461	0·0037
Paris I			
Inadequate response vs. no UDCA	0·556	0·448-0·690	<·0001
Response vs. no UDCA	0·291	0·240-0·357	<·0001
Barcelona			
Inadequate response vs. no UDCA	0·511	0·415-0·630	<·0001
Response vs. no UDCA	0·316	0·262-0·387	<·0001

P-values were assessed using an IPTW-adjusted Cox proportional hazard model.

[a] The stratified Hazard Ratios were adjusted for sex, age, year of diagnosis, albumin, platelet count, bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase.

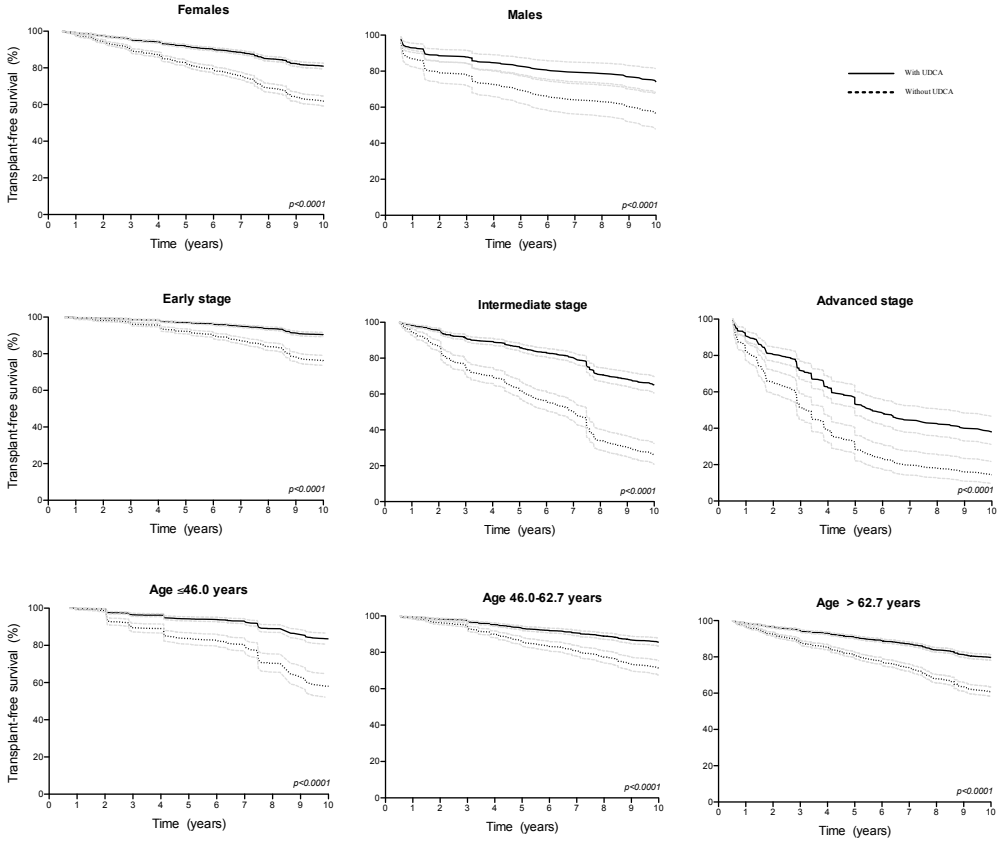
Abbreviations: UDCA, ursodeoxycholic acid; HR, hazard ratio; CI, confidence interval.

Supplementary Figure S1. Baseline characteristics stratified for treatment after inverse probability of treatment weighting adjustments. The boxplots represent the median and interquartile range, the whiskers represent the 5th and 95th percentiles.



Balance assessment by comparing treated and untreated patients after inverse probability of treatment weighting.
Abbreviations: UDCA, ursodeoxycholic acid; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ULN, upper limit of normal; LLN, lower limit of normal.

Supplementary Figure S2. Transplant-free survival according to UDCA treatment, stratified for baseline characteristics. The solid line represents the weights-adjusted survival of UDCA-treated patients, the dotted line reflects the weights-adjusted survival of untreated patients. The 95% confidence intervals are reflected by the grey lines



The survival figures were constructed using IPTW-adjusted Cox proportional hazard models.
Abbreviations: UDCA, ursodeoxycholic acid; IPTW, inverse probability of treatment weighting.

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CHAPTER 5
THE NUMBER NEEDED TO TREAT WITH URSODEOXYCHOLIC ACID TO PREVENT LIVER TRANSPLANTATION OR DEATH IN PRIMARY BILIARY CHOLANGITIS

Gut, 2019

ABSTRACT

Objectives Clinical benefit of ursodeoxycholic acid (UDCA) in primary biliary cholangitis (PBC) has never been reported in absolute measures. The aim of this study was to assess the number needed to treat (NNT) with UDCA to prevent liver transplantation (LT) or death among patients with PBC.

Methods The NNT was calculated based on the untreated LT-free survival and hazard ratio's (HR) of UDCA with respect to LT or death as derived from inverse probability of treatment weighting-adjusted Cox proportional hazard analyses within the Global PBC Study Group database.

Results We included 3902 patients with a median follow-up of 7.8 (4.1-12.1) years. The overall HR of UDCA was 0.46 (95%CI 0.40-0.52) and the 5-year LT-free survival without UDCA was 81% (95%CI 79-82). The NNT to prevent one LT or death within 5 years (NNT_{5Y}) was 11 (95% CI 9-13). Although the HR of UDCA was similar for patients with and without cirrhosis (0.33 vs. 0.31), the NNT_{5y} was 4 (95%CI 3-5) and 20 (95%CI 14-34), respectively. Among patients with low ALP (≤2x the upper limit of normal [ULN]), intermediate ALP (2-4x ULN) and high ALP (>4x ULN) the NNT_{5y} to prevent one LT or death was 26 (95%CI 15-70), 11 (95%CI 8-17), and 5 (95%CI 4-8), respectively.

Conclusion In this large international PBC cohort, the absolute clinical efficacy of UDCA with respect to LT or death varied with baseline prognostic characteristics, but was high throughout. These findings strongly emphasize the incentive to promptly initiate UDCA treatment in all patients with PBC and may improve patient compliance.

INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic disease of the liver, characterized by destruction of the small intrahepatic bile ducts and formation of hepatic fibrosis[1, 2]. It was recently estimated that nowadays 40% of the patients with PBC will develop cirrhosis within 10 years, at which point patients are at increased risk of liver failure and hepatocellular carcinoma[3]. As a result, the overall survival of patients with PBC is substantially impaired as compared to that of a matched general population[4].

The choleretic and hydrophilic bile acid ursodeoxycholic acid (UDCA) is currently considered as the standard of care for patients with PBC[5-7]. Based on long-term clinical experience, UDCA is considered to have a favorable safety profile. The strong association between UDCA therapy and prolonged liver transplantation (LT)-free survival was recently substantiated in both a large American cohort and our own international cohort, with a dose-response relationship highlighting the importance of the 13-15 mg/kg dose recommendation[8]. Still, even in recent Western cohorts as much as 30% of patients remained untreated and suboptimal UDCA dosages were frequently used[9, 10]. More awareness of and attention for the clinical efficacy of UDCA is thus needed in order to optimize the medical management and clinical outcome of the population with PBC.

While previous studies only assessed the relative reduction of the risk of clinical outcomes with UDCA therapy, our understanding of the impact of UDCA could benefit from reports of absolute measures of clinical efficacy. The number needed to treat (NNT) to prevent one clinical event represents such an absolute clinical efficacy measure with clear interpretation for physicians, patients and policymakers. Currently, it is not known how many patients with PBC should be treated with UDCA to prevent one LT or death. Although previously we showed that the relative risk reduction with UDCA is stable over various patient characteristics, the absolute risk reduction may not be[8]. In this study we aimed to assess the NNT with UDCA to prevent one LT or death among patients with PBC. Secondary aims were to evaluate the NNT in various subgroups of patients with PBC and to estimate the NNT for the individual PBC patient.

PATIENTS AND METHODS

Study population and design

For the current study we used the data of patients included in the database of the Global PBC Study Group, which is an international collaboration between liver units across 8 countries in Europe and Northern America. The database contains data from representative long-term followed cohorts on an individual patient level of both UDCA-treated and untreated patients. All patients had an established diagnosis of PBC according to the internationally accepted guidelines[6, 7]. Patients were only included in case of sufficient follow up (>6 months and ≥2 recorded visits), and when dates of starting UDCA treatment and/or clinical events were known. For the current analyses we excluded patients in case an auto-immune overlap syndrome or other concomitant liver disease was present. Further details on the methodology of data collection have been described in further detail elsewhere[8, 12]. In line with our previous work, 3902 patients were included for the current analyses[8]. This study was conducted in accordance with 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 their local regulations.

Statistical analysis

The outcome measure of the current study was the combined endpoint of LT and all-cause mortality. Baseline was considered to be the first center visit in untreated patients and the start treatment in patients receiving UDCA. Treatment with UDCA for PBC is recommended lifelong and usually initiated promptly after diagnosis. In our study the median (interquartile range [IQR]) interval between the first center visit and start of UDCA was 2.9 months (0-29). Patients were censored at time of LT or at time of their last center visit in case no events occurred during the follow-up. Missing baseline data was assumed to be missing at random and was handled by means of multiple imputation *(SAS Proc MI, MCMC method)*. Hereto, 10 databases were generated with use of Rubin's rules to estimate the parameters and the standard error. The biochemical values included for imputation were: alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, and platelet count. Categorical or binary variables were not imputed.

Because treatment was not assigned randomly in our study population, our analyses were performed following inverse probability treatment weighting (IPTW)[13]. Hereto, following stabilization, weights were assigned to each individual patient based on the predictive values derived from a logistic regression model including baseline patient characteristics and laboratory parameters (age, gender, calendar year of diagnosis, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), platelet count, albumin) with UDCA therapy as dependent variable[8, 14]. After weighting a balance assessment was performed which previously showed that there were no remaining differences in baseline characteristics

between the UDCA-treated and untreated patients[15]. Subsequently, the association between time to LT or death and UDCA therapy was assessed through Cox proportional hazard regression analyses.

The NNT to prevent one LT or death within [t] years with UDCA therapy can be calculated with the LT-free survival in patients without treatment and the estimated benefit of UDCA on LT-free survival, which are both derived from IPTW-adjusted Cox regression analyses. The NNTs were estimated using the following formula: NNT = (1/(LT-free survival$_{untreated}$[t]$^{HR\ UDCA}$)-(LT-free survival$_{untreated}$[t])).[16] The 95% confidence intervals (CI) of both the LT-free survival and the HR of UDCA were taken into account to address the uncertainty of the NNT. Unrounded numbers of HR and untreated survival were used to calculate the NNT. The NNT was always rounded up. Although the NNT to prevent one LT or death can be calculated for every time point [t] during the follow-up (NNT$_{[t]y}$), we primarily report the NNT to prevent one LT or death within 5 years (NNT$_{5y}$) throughout the manuscript. Stratified analyses were performed based on categorized baseline characteristics.

The individualized NNT$_{5y}$ was estimated with use of the GLOBE score, a validated objective prognostic tool which was developed to accurately assess the LT-free survival after 1 year of UDCA therapy. The GLOBE score is calculated with the formula: 0.044378 x age + 0.93982 x LN(bilirubin) + 0.335648 x LN(ALP) + 2.266708 x albumin + 0.002581 x platelets (per 10^9/L) + 1.216865 (bilirubin and alkaline phosphatase in 'x upper limit of normal' and albumin in 'x lower limit of normal')[17]. First, the predictive accuracy of the GLOBE score (calculated with the variables at baseline) for LT or death was assessed in untreated patients using the c-statistic[18, 19]. Calibration analyses were performed by comparing the predicted mortality rates with those observed. Second, a multivariable Cox regression model for LT or death including the GLOBE was constructed. Linearity was assessed by including polynomial terms, which remained included in the multivariate model in case these were statistically significantly associated with the outcome measure. Subsequently, the HR of UDCA was calculated for each value of the GLOBE score.

All statistical tests were two-sided, and a P-value <0.05 was considered to be statistically significant. The significance level for interactions was set at p<0.01 to correct for multiple testing. Statistical analyses were performed in SPSS Statistics V.21.0 (Armonk, NY: IBM Corp.) and SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Cohort characteristics

Included in the study were 3902 patients with PBC, predominantly female (91.0%) and with a mean (standard deviation) age of 54.3 (11.9). Treated with UDCA were 3529 (90.4%) patients and not treated with UDCA were 373 (9.6%) patients. **Table 1** shows the baseline characteristics according to the treatment with UDCA prior to IPTW. Following adjustment with IPTW there were no remaining baseline characteristics which differed statistically significantly between the two groups. Patients were followed for a median during of 7.8 (IQR 4.1-12.1) years during which a total of 299 patients underwent LT and 567 patients died. The primary endpoint of LT or death was observed in 721 UDCA-treated patients and 145 untreated patients.

NNT_{5y} with UDCA to Prevent One LT or Death

Following IPTW adjustment, the 5-year cumulative LT-free survival without UDCA therapy was 81.0% (95% CI 79.3 – 82.7). The overall adjusted HR of UDCA for LT or death was 0.46 (95%CI 0.40-0.52, p<0.001). As a result, the NNT_{5y} to prevent LT or death in one patient was 11 (95% CI 9-13). With a proportional HR of UDCA over time, the cumulative LT-free survival in untreated patients at (t) years drives the estimated NNT to prevent one LT or death over that specific duration of therapy.

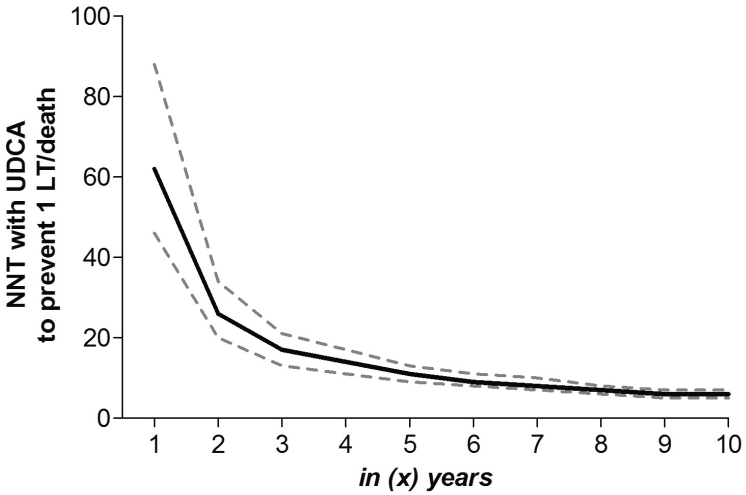

Figure 1. Adjusted NNT to prevent one LT or death according to the treatment duration with 95% CI.
With a 10-year cumulative LT-free survival of 60.7% (95% CI 58.2 – 63.4) in absence of UDCA, the NNT10y to prevent one LT or death was 6 (95%CI 5-7). *Figure 1* shows the NNT(t)y to prevent one LT or death according to various durations of UDCA therapy.
Abbreviations: CI, confidence interval; LT, liver transplantation; NNT, number needed to treat; UDCA, ursodeoxycholic acid.

Table 1. Baseline characteristics

	Overall N = 3902	UDCA-treated N = 3529	Untreated N = 373	P value
Age at diagnosis, years[a]	52.3 (11.9)	52.1 (11.7)	54.1 (13.4)	<0.001
Female, n (%)	3552/3902 (91.0)	3209/3529 (90.9)	343/373 (92.0)	0.510
AMA positive, n (%)	3507/3862 (90.8)	3175/3491 (90.9)	332/371 (89.5)	0.418
Year of diagnosis[b]	1996 (1990-2003)	1997 (1990-2003)	1992 (1982-2000)	<0.001
Histological disease stage, n (%)[c]				<0.001
Stage I	784/2173 (36.1)	739/2076 (35.6)	45/97 (46.4)	
Stage II	671/2173 (30.9)	657/2076 (31.6)	14/97 (14.4)	
Stage III	365/2173 (16.8)	351/2076 (16.9)	14/97 (14.4)	
Stage IV	353/2173 (16.2)	329/2076 (15.8)	24/97 (24.7)	
Serum bilirubin (ULN)[b]	0.63 (0.44-1.00)	0.62 (0.44-1.00)	0.65 (0.43-1.38)	0.081
Serum ALP (ULN)[b]	2.29 (1.41-3.95)	2.32 (1.46-4.00)	1.94 (1.11-3.51)	<0.001
Serum AST (ULN)[b]	1.53 (1.03-2.31)	1.56 (1.05-2.34)	1.25 (0.75-2.00)	<0.001
Serum ALT (ULN)[b]	1.68 (1.05-2.63)	1.71 (1.09-2.68)	1.20 (0.75-1.83)	<0.001
Serum albumin (LLN)[b]	1.15 (1.06-1.25)	1.15 (1.06-1.25)	1.15 (1.03-1.26)	0.840
Platelet count (x10^3/mm^3)[b]	245 (190-300)	248 (195-303)	217 (146-271)	<0.001
Biochemical disease stage, n (%)[d]				<0.001
Early	1576/2296 (68.6)	1376/1980 (69.5)	200/316 (63.3)	
Advanced	559/2296 (24.3)	484/1980 (24.4)	75/316 (23.7)	
Severe	161/2296 (7.0)	120/1980 (6.1)	41/316 (13.0)	

Serum bilirubin was missing for 1020 (26%) patients, serum ALP for 1069 (27%), serum AST for 1175 (30%), serum ALT for 1294 (33%), serum albumin for 1533 (39%), and platelet count for 1720 (44%), AMA status was missing for 40 (1.9%).

[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[20];

[d] Biochemical disease stage according to Rotterdam criteria[21].

Abbreviations: AMA, antimitochondrial antibodies; ALP, alkaline phosphatase; ULN, upper limit of normal; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LLN, lower limit of normal; UDCA, ursodeoxycholic acid.

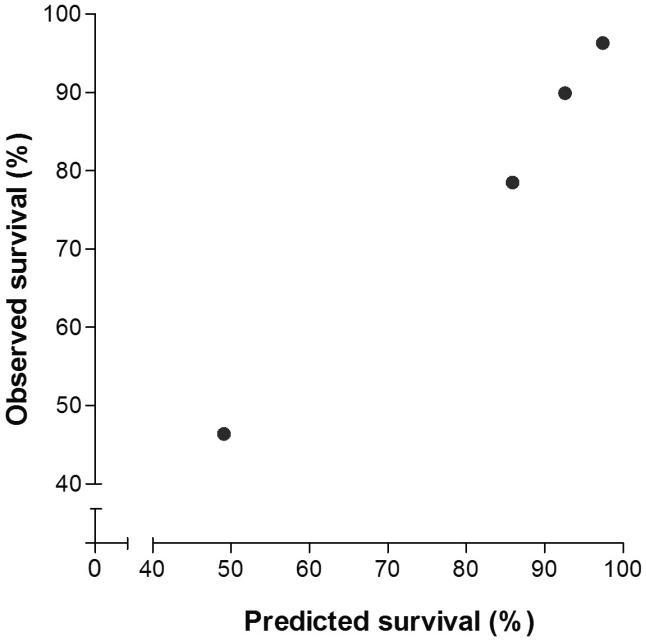

Figure 2. Observed versus predicted LT-free survival according to categorized risk groups. The observed and mean predicted liver LT-free survival of patients in our cohort with: 1) a GLOBE score ≤ -0.21, i.e. 5-year risk of LT/death ≤ 5%; 2) GLOBE 0.21 - 0.51, i.e. 5-year risk 5-10%; 3) GLOBE 0.51 - 0.91 i.e. 5-year risk of 10-20%; and 4) a GLOBE score of > 0.91, corresponding with a 5-year risk of >20%. Abbreviation: LT, liver transplantation.

Relative Risk Reduction versus Absolute Risk Reduction in Stratified Subgroups

Table 2 presents the adjusted HRs of UDCA with respect to LT or death, the adjusted cumulative LT-free survival in UDCA-untreated patients and the adjusted NNT to prevent one LT or death within 5 and 10 years for various subgroups of patients. As previously described, the HR of UDCA for LT or death was stable over the baseline characteristics and only differed statistically significantly among patients stratified according the their baseline age, and ALP and albumin levels[8]. As example, the relative reduction of the risk of LT or death with UDCA therapy was somewhat - although not statistically significantly - lower among patients with early biochemical disease (adjusted HR 0.37, 95%CI 0.30-0.47) or patients intermediate biochemical disease (adjusted HR 0.32, 95%CI 0.25-0.40) versus patients with advanced biochemical disease (adjusted HR 0.50, 95%CI 0.37-0.70). In absolute terms, however, the adjusted NNT_{5y} to prevent one LT or death was substantially higher among those with early biochemical disease (22, 95%CI 17-32) as opposed to those with intermediate or advanced disease (5 [95%CI 4-6] and 5 [95%CI 3-8], respectively). The beneficial NNT in patients with advanced biochemical response is explained by the higher 5-year cumulative incidence of LT or death (26.2% [95%CI 20.4-33.7]). The IPTW-adjusted HR of UDCA was statistically significantly stronger among the youngest quartile of patients (≤46.0 years; 0.33 [95%CI

Table 2. The NNT with UDCA to prevent one LT or death in 5 and 10 years in subgroups of patients with PBC

Characteristic	aHR (95%CI)[a]	p-value HR	Untreated LT-free survival5y (95%CI)	NNT5y (95%CI)	Untreated LT-free survival10y (95%CI)	NNT10y (95%CI)
Sex						
Males	0.52 (0.35-0.77)	0.0011	0.68 (0.60-0.76)	**8 (5-21)**	0.55 (0.46-0.64)	**6 (4-15)**
Females	0.44 (0.38-0.52)	<.0001	0.82 (0.80-0.84)	**11 (9-14)**	0.62 (0.59-0.64)	**6 (5-7)**
Age (years)						
≤46.0	0.33 (0.24-0.46)	<.0001	0.83 (0.79-0.86)	**9 (7-14)**	0.60 (0.55-0.66)	**5 (3-6)**
46.0-62.7	0.46 (0.37-0.56)	<.0001	0.80 (0.78-0.83)	**10 (8-14)**	0.67 (0.64-0.71)	**7 (5-9)**
>62.7	0.60 (0.48-0.76)	<.0001	0.81 (0.77-0.84)	**14 (9-28)**	0.52 (0.47-0.58)	**7 (5-13)**
Cirrhosis[b]						
No	0.32 (0.24-0.42)	<.0001	0.92 (0.90-0.95)	**20 (14-34)**	0.71 (0.66-0.76)	**6 (5-8)**
Yes	0.31 (0.24-0.40)	<.0001	0.48 (0.42-0.54)	**4 (3-5)**	0.33 (0.27-0.39)	**3 (3-4)**
Disease stage[c]						
Early	0.37 (0.30-0.47)	<.0001	0.92 (0.91-0.94)	**22 (17-32)**	0.78 (0.75-0.80)	**8 (6-11)**
Intermediate	0.32 (0.25-0.40)	<.0001	0.62 (0.57-0.67)	**5 (4-6)**	0.22 (0.17-0.28)	**3 (3-4)**
Advanced	0.50 (0.37-0.70)	0.0001	0.26 (0.20-0.34)	**5 (3-8)**	0.14 (0.92-0.20)	**5 (4-9)**
ALP						
≤ 2x ULN	0.61 (0.45-0.82)	0.0014	0.90 (0.87-0.92)	**26 (15-70)**	0.79 (0.75-0.82)	**13 (8-35)**
2-4x ULN	0.46 (0.36-0.59)	<.0001	0.82 (0.79-0.85)	**11 (8-17)**	0.59 (0.56-0.64)	**6 (4-8)**
> 4x ULN	0.36 (0.25-0.52)	<.0001	0.66 (0.62-0.70)	**5 (4-8)**	0.41 (0.36-0.46)	**4 (3-5)**
Bilirubin						
≤ ULN	0.39 (0.32-0.48)	<.0001	0.91 (0.90-0.92)	**19 (15-27)**	0.75 (0.72-0.78)	**7 (6-10)**
> ULN	0.40 (0.33-0.48)	<.0001	0.49 (0.45-0.53)	**4 (4-5)**	0.20 (0.16-0.25)	**4 (3-4)**
Albumin						
< LLN	0.32 (0.24-0.43)	<.0001	0.35 (0.29-0.41)	**3 (3-4)**	0.15 (0.11-0.21)	**3 (3-4)**
≥ LLN	0.46 (0.40-0.54)	<.0001	0.87 (0.86-0.89)	**16 (13-21)**	0.68 (0.66-0.71)	**7 (6-9)**
Platelet count						
< 150x109	0.48 (0.35-0.46)	0.0007	0.52 (0.47-0.58)	**5 (4-9)**	0.27 (0.22-0.34)	**4 (3-7)**
≥ 150x109	0.44 (0.37-0.52)	<.0001	0.86 (0.84-0.87)	**14 (11-18)**	0.68 (0.65-0.70)	**7 (5-8)**

[a] The Hazard Ratios were adjusted for sex, age, year of diagnosis, albumin, platelet count, bilirubin, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase;
[b] Baseline histological data was available for 2173 patients;
[c] Biochemical disease stage according to Rotterdam criteria[29];
Abbreviations: 5y, 5 years; 10y, 10 years; adj., adjusted; ALP, alkaline phosphatase; CI, confidence interval; HR, hazard ratio; LLN, lower limit of normal; LT, liver transplantation; NNT, number needed to treat; PBC, primary biliary cholangitis; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

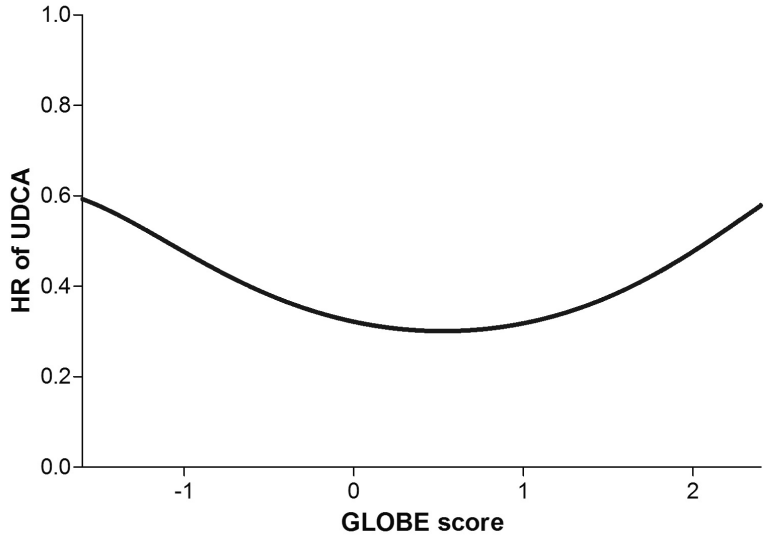

Figure 3. HR of UDCA on LT-free survival according to the GLOBE score. The graph shows a non-linear relationship, in which the function of the GLOBE score was significant to the fourth degree.
Abbreviations: HR, hazard ratio; UDCA, ursodeoxycholic acid.

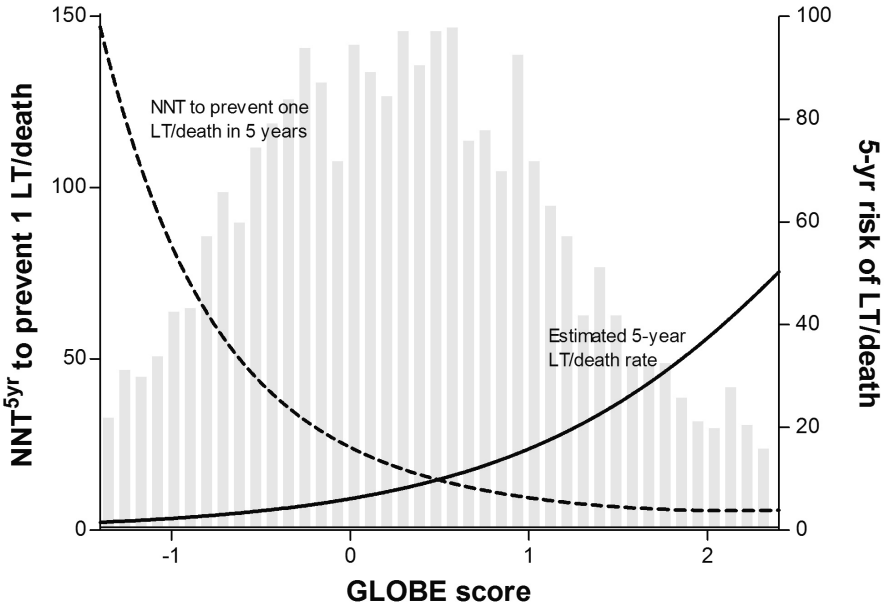

Figure 4. Individualized NNT5y according to the GLOBE score, visualized against the estimated 5-year risk of liver transplantation or death. The solid line represents the estimated 5-year risk of LT or death, plotted against the right Y-axis. The dotted line represents the number needed to treat for 5 years to prevent the occurrence of one LT or death, plotted against the left Y-axis. The grey bars represent a histogram of the number of patients in our cohort according to their GLOBE score, plotted against the left Y-axis.
Abbreviations: LT, liver transplantation; NNT, number needed to treat.

0.24-0.46]) as compared to those in the interquartile age range (46.0-62.7 years; 0.46 [95%CI 0.37-0.56]) and the oldest quartile of patients (>62.7 years; 0.60 [95%CI 0.48-0.76]), while the cumulative 5-year LT-free survival rates without UDCA were rather similar among the three age groups. The stronger adjusted HR of UDCA among patients ≤46 years resulted in an adjusted NNT_{5y} to prevent one LT or death of 9 [95%CI 7-14), which was lower as compared to 10 (95%CI 8-14) in those aged 46.0-62.7 years and 14 (95%CI 9-28) in those older than 62.7 years.

Predicted Individual NNT to prevent one liver transplantation or death

In the untreated population, the discriminative ability of the GLOBE score was strong with a C-statistic of 0.81 (95% CI 0.78 - 0.85). The observed 5-year transplant-free survival was in line with the predicted estimates using the GLOBE score (**Figure 2**). **Figure 3** shows the polynomial function of the HR of UDCA according to the GLOBE score, which was significant to the fourth degree. Using the estimated 5-year survival that relates to every value of the GLOBE score, we predicted the NNT5y for any given GLOBE score (**Figure 4**). A NNT5y ≤10 to prevent one LT/death is achieved in patients with a GLOBE score ≥0.94 (NNT10y=5), a NNT5y of 20 in patients with a GLOBE score of 0.10 (NNT10y=9), while the NNT5y is ≥50 in case the GLOBE score is <-0.62 (NNT10y=20).

Relative Risk Reduction versus Absolute Risk Reduction According to Biochemical Response

In our cohort, 2084 (59.1%) UDCA-treated patients had an ALP<1.67 xULN at year 1, and their 5- and 10-year LT-free survival rates were 94.0% and 84.7%. These patients had a lower risk of LT or death (adjusted HR 0.35, 95%CI 0.29-0.42, p<0.0001) as opposed to those without UDCA. In contrast, 1445 (40.9%) patients had an suboptimal biochemical response. In these patients the 5- and 10-year LT-free survival rates were 88.0% and 70.9%. Although less strong, a suboptimal response to UDCA remained associated with a statistically significantly lower risk of LT or death as compared to no UDCA (adjusted HR 0.42, 95%CI 0.36-0.50, p<0.0001). Among patients with an ALP<1.67 xULN, the median GLOBE score prior to UDCA treatment was -0.0266, which translates into estimated 5- and 10-year LT-free survival rates of 94.0% and 84.7%. As a result, the NNT was 26 (95%CI 24-29) and 11 (95%CI 10-12) to prevent 1 LT or death in 5 or 10 years, respectively. In contrast, the median GLOBE score prior to UDCA treatment was 0.6978 among patients with a suboptimal biochemical response, leading to estimated 5- and 10-year LT-free survival rates of 88.0% and 70.9%. As a result, their NNT5y was 15 (95%CI 14-18) and the NNT10y was 7 (95%CI 6-8).

DISCUSSION

In our large international cohort, the overall number of patients with PBC which needed to be treated with UDCA to prevent one LT or death within five years was 11, as the related relative risk reduction was 2.2 and the cumulative 5-year incidence of LT/death in untreated patients was approximately 19%. This NNT, as absolute measure of clinical efficacy, further decreased in case one LT/death had to be prevented over longer periods of time. This is relevant, as UDCA is recommended as lifelong therapy for patients with PBC. The NNT fluctuated according to the baseline patient characteristics, which is predominantly explained by differences in the natural history of PBC in various subgroups. Nevertheless, the clinical efficacy of UDCA in terms of the number needed to treat to postpone one LT or death with at least five years can be considered low throughout.

In the current study, the NNT was assessed across all relevant patient subgroups. We previously found that the relative reduction in the risk of LT/death associated with UDCA was generally stable[8]. For instance, the HR of UDCA was similar among patients with cirrhosis (HR 0.33) and patients without cirrhosis (HR 0.31). However, the absolute clinical efficacy of UDCA was considerably lower among patients with cirrhosis (NNT_{5y} 4) as compared to those without cirrhosis (NNT_{5y} 20). This difference is explained by the substantially higher cumulative 5-year incidence of LT/death in untreated patients with cirrhosis (52%) than in those without cirrhosis (7%). This emphasizes the relevance of appreciating the clinical setting when evaluating the clinical benefit of a therapeutic intervention, which is considered when using the NNT as a measure of efficacy. The relative risk reduction associated with UDCA with respect to LT/death did differ according to ALP, age and albumin[8]. ALP is an established prognostic marker for long-term outcome[12, 22]. Among patients with a high ALP level (>4 ×ULN) the HR of UDCA was stronger and the cumulative 5-year incidence of LT/death in absence of treatment was higher in comparison to patients with lower ALP levels. Both factors contributed to the considerably lower NNT_{5y} to prevent LT/death in patients with high ALP (5) than in those with low ALP levels (≤2 ×ULN: 26) before the initiation of UDCA. Young age was associated with a stronger relative risk reduction related to UDCA treatment. Although younger age is normally inversely associated with the risk of death, patients that develop PBC at young age are known to have a more aggressive phenotype[23]. Indeed, the cumulative LT-free survival among untreated PBC patients ≤46 years in our cohort was similar as compared to that of older patient subgroups. As a result, the NNT_{5y} was only slightly lower in patients ≤46 years (9) as compared to patients aged 46-63 years (10) and >63 (14). In line with the above, the absolute clinical efficacy of UDCA therapy was stronger among patients with a suboptimal biochemical response at year 1, despite an inferior relative risk reduction. Although this might seem counterintuitive, this is explained by the impaired untreated LT-free survival in these patients when compared to those with an ALP < 1.67 xULN after year 1.

As exemplified in the previous paragraph, the untreated prognosis strongly affects the absolute clinical efficacy of UDCA. For an individual patient, multiple baseline characteristics need to be considered, while it would be desirable to estimate a single patient-specific NNT. We showed that the GLOBE score, originally developed as an objective tool to estimate LT-free survival after 1 year on UDCA treatment, also accurately predicts prognosis in untreated patients. Hereafter, we estimated the individualized clinical efficacy of UDCA according to the GLOBE score. In this analysis we allowed the HR of UDCA to fluctuate with the GLOBE score as it incorporates ALP and age, the two variables with most profound and significant impact on the relative risk reduction of UDCA. An estimation of an individual NNT can be helpful for patient counseling and supporting therapeutic compliance. For example, patients might be more willing to accept perceived side effects due to an improved understanding of the expected absolute risk reduction. Noteworthy is that a high NNT with UDCA was usually a result of a favorable natural history rather than the absence of a relative benefit of UDCA.

To the best of our knowledge, this is the first study to assess the benefit of UDCA treatment in PBC in absolute risk reduction as measured by the NNT to prevent clinical endpoints. Assessment of the NNT is rare in the field of hepatology, but has recently gained popularity in many other fields of medicine. The advantage of the NNT is that it is easy to interpret for both patients and physicians as it combines the therapy-induced relative risk reduction and the patients' a priori risk of unfavorable outcome in a single parameter. The NNT can be expressed for any given treatment duration, which is especially relevant for chronic diseases such as PBC in which lifelong treatment is required. While policymakers may be interested in long-term effects of therapy, patients are more likely to prioritize short-term benefits. Moreover, physicians' willingness to treat is reported to be dependent of the measure in which treatment benefit is presented. Providing information on both relative and absolute clinical efficacy may therefore prevent misinterpretation and aid well-informed decision making in daily clinical practice[24-26]. As part of our study we validated the GLOBE score to accurately predict the LT-free survival in untreated patients with PBC. The availability of such an objective natural history score is relevant, also in light of novel second-line therapies which will no longer be compared with a placebo arm given the strong evidence for a beneficial effect of UDCA for all patients with PBC[8]. The GLOBE score can thus aid to evaluate the potential additional benefit of new drugs that are added to treatment with UDCA, and might be preferable over older prediction models such as the Mayo Risk Score as it is solely based on readily available and objective parameters[27].

Strengths of the current study include the use of a large, internationally representative cohort with long-term follow-up and many clinical endpoints in both UDCA-treated and untreated patients. Furthermore, to ensure accurate estimation of the NNT, both the 95% CI of the HR of UDCA as well as the CI of the estimated survival in the untreated population. A number of limitations should also be noted. First, a potential selection bias in this study

is represented by the fact that the majority of included patients were treated in tertiary liver centers. Second, potential improvement in survival within the timespan that is chosen to express the NNT is not considered when using the NNT as measure of risk reduction, which could thus lead to an underestimation of treatment benefit. Third, the NNT assumes a causal relationship between UDCA and prolonged LT-free survival. This has long been subject to debate, especially due to Cochrane reporting an absence of treatment benefit[28]. As this is a retrospective study in which IPTW was used to adjust for the small differences in baseline characteristics, residual confounding can never be fully ruled out. We are lacking data on the reasons for not treating PBC patients with UDCA, but especially shortly after its introduction it can be hypothesized that physicians may have been unaware of UDCA or not convinced about its benefits. Also, patients may have been unwilling to use this relatively new drug at that time. Because UDCA has no relevant contra-indications, however, we consider it to be unlikely that the association between UDCA and improved LT-free survival is completely confounded by a patient-related factor which would have influenced both the chance of receiving UDCA and the risk of LT or death. In fact, both the positive association with clinical outcome in extensively adjusted analyses in large cohort studies, and the finding of an improved LT-free survival in UDCA-treated patients with advanced disease in an older randomized controlled trial, have provided a general consensus on the assumed causal UDCA treatment benefit[6-8, 29].

In conclusion, in this first study to assess the efficacy of UDCA in absolute measures, we report that the NNT with UDCA to prevent LT or death is generally low, but can be assessed for individual patients with PBC. These results provide a clear understanding of the clinical importance of optimized UDCA therapy for patients and doctors, thereby stimulating compliance and treatment uptake.

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Hepatol. Commun. 2018 Apr 19;2(6):683-692.

CHAPTER 6
CLINICAL APPLICATION OF THE GLOBE SCORE AND UK-PBC SCORE IN A REAL WORLD TRIAL COHORT OF PATIENTS WITH PRIMARY BILIARY CHOLANGITIS

Hepatology Communications, 2018

ABSTRACT

Introduction The Global Primary Biliary Cholangitis (PBC) Study Group and United Kingdom-PBC (UK-PBC) Consortium have demonstrated that dichotomous response criteria are not as accurate as continuous equations at predicting mortality or liver transplantation in PBC. The aim of this analysis was to assess the clinical utility of the GLOBE and UK-PBC risk scores using data from POISE, a phase 3 trial investigating obeticholic acid (OCA) in patients with PBC.

Methods Data (N 5 216) at baseline and month 12 were used to calculate the GLOBE and UK-PBC risk scores to assess the projected change in risk with OCA versus placebo. Additionally, the benefit of OCA was assessed in patients not meeting the POISE primary endpoint.

Results Both the GLOBE and UK-PBC risk scores predicted a significant reduction in long-term risk of death and liver transplantation after OCA treatment ($P < 0.0001$). The differences in the relative risk reduction from baseline in the 10-year event risk after 1 year for OCA 10 mg versus placebo was 26% (GLOBE) and 37% (UK-PBC). The scores also predicted a significantly decreased risk in patients treated with OCA who did not meet POISE response criteria after 1 year of treatment compared to an increased risk with placebo ($P < 0.0001$).

Conclusion This analysis demonstrates the use of the GLOBE and UK-PBC risk scores to assess risk reduction of a cohort treated with OCA. While validation of this risk reduction in studies with clinical outcomes is needed, this study highlights the potential use of these scores in individualizing risk prediction in PBC both in clinical practice and therapeutic trials.

INTRODUCTION

In primary biliary cholangitis (PBC), progression of liver disease is highly variable[1,2].In many cases, the disease is detected at an early stage and treatment with ursodeoxycholic acid (UDCA) improves biochemistry, impedes hepatic fibrosis, and can restore normal life expectancy[3-5]. However, in a substantial proportion of patients, the response to treatment with UDCA is inadequate. These patients experience progressive liver disease that may eventually lead to liver failure or hepatocellular carcinoma[6]. It is well established that the liver biochemistry on treatment with UDCA strongly predicts long-term outcomes in PBC[7-11].

The response to treatment with UDCA (so-called UDCA response) may therefore be defined in terms of the liver biochemistry measured at a specific time (usually 12 months) after starting treatment. Several definitions of the UDCA response have been proposed[7-11]. One of these definitions was the Toronto criteria, and a composite of Toronto along with other criteria were used to define treatment response criteria for POISE, a phase 3 trial of treatment with obeticholic acid (OCA) reported by Nevens et al [12]. By these criteria, response was defined as alkaline phosphatase (ALP)) <1.67 x the upper limit of normal (ULN), 15% reduction in ALP, and total bilirubin ULN. For prognostication, definitions of UDCA response, such as the Toronto, Paris, Rotterdam, and Barcelona criteria, have two major limitations: first, they dichotomize UDCA response and thereby the long-term risk of death or liver transplantation (LT), whereas both are, in reality, a continuum; second, they do not account for the stage of disease. Two independent research groups, the Global PBC Study Group and the United Kingdom (UK)-PBC Consortium, developed and externally validated continuous prognostic models (the GLOBE score and UK-PBC risk score, respectively) that address these limitations[13,14]. These models include the liver biochemistry following treatment with UDCA as well as surrogate measurements of disease stage (e.g., serum albumin and platelet count). They estimate the risk of LT or death (overall death for the GLOBE score and liver-related death for the UK-PBC risk score) in patients with PBC at specific time points. Both scores outperformed previous response criteria[7-11,13,14] in terms of prognostic utility and could potentially help physicians identify patients at high risk of disease progression and in need of second-line therapy. They have also been validated in patients not treated with UDCA, strongly suggesting that such scoring systems reflect disease activity and stage expressed by the laboratory investigations, regardless of treatment. Recently published guidelines from the European Association for the Study of the Liver propose these criteria as tools to select patients for second-line therapies and possibly for a better design of clinical trials in PBC in the future[15].

The aim of this study was to explore the utility of the GLOBE score and UK-PBC risk score in a trial data set comprising individual patient data from the phase 3 POISE trial of OCA.

In addition, we use validated risk scores to evaluate the predicted risk reduction of OCA therapy in patients with PBC who inadequately respond to UDCA.

METHODS

Study population

The patient demographics and study design of the POISE trial were reported by Nevens and colleagues[12] (**Table 1**). Briefly, POISE was a phase 3, randomized, double-blind, placebo-controlled trial. PBC diagnosis was defined by American Association for the Study of Liver Diseases and European Association for the Study of the Liver guidelines[15,16]. Patients were recruited across 13 countries. All patients were over 18 years old, met the study entry criteria of ALP 1.67 3 ULN and/or total bilirubin >ULN but 0.80). Similar results were found when the score was validated in an untreated population, with a C statistic of 0.81.

GLOBE score

The GLOBE score has recently been validated externally[17].

GLOBE score =
0.044378 x age at start of UDCA therapy + 0.93982 x ln(bilirubin x ULN at 1 year follow-up) + 0.335648 x ln(ALP x ULN at 1 year follow-up) – 2.266708 x albumin level x the lower limit of normal (LLN) at 1 year follow-up - 0.002581 x platelet count per 10^9 /L at 1 year follow-up + 1.216865.

UK-PBC risk score

Derivation of the UK-PBC risk score has been described in detail[13]. The score was developed based on 1,916 UDCA-treated participants from the UKPBC Research Cohort. The final model, shown below, consisted of the baseline albumin and platelet count as well as total bilirubin, transaminases, and ALP after 12 months of UDCA treatment. Linear predictors and baseline survivor functions were combined in equations to score the risk of an LT or liver-related death occurring within 5, 10, or 15 years. The risk score was validated in an independent cohort of 1,249 UDCA-treated participants from the UK-PBC Research Cohort. In the validation cohort, the 5-, 10-, and 15-year risk scores were highly accurate (C statistic >0.90). Similar results were found when the score was validated in an untreated population from the United Kingdom. The UK-PBC risk score has recently been validated externally[17].

UK-PBC risk score =
1 – baseline survival function^exp(0.0287854 x [ALP after 12 months of therapy x ULN – 1.722136304] – 0.0422873 x [((ALT where this was available, otherwise AST, after 12 months of therapy x ULN/10)^–1) – 8.675729006] + 1.4199 x [ln(bilirubin after 12 months of

therapy x ULN/10) + 2.709607778] – 1.960303 x [albumin at baseline x LLN – 1.17673001] – 0.4161954 x [platelet count at baseline x LLN –1.873564875]).

Statistical analyses

Individual patient data at baseline and month 12 from the POISE trial were used to calculate both scores to compare the projected improvement in risk/ survival after 1 year of OCA treatment versus placebo in patients enrolled in the POISE trial. The P value for comparing each OCA treatment to placebo is obtained using the rank analysis of covariance model with baseline value as a covariate. Individual baseline values were based on a mean of all available study evaluations prior to OCA treatment or placebo. In order to evaluate the change in risk using the GLOBE score, the participant's contemporaneous age was used in place of his or her age at the start of UDCA therapy. $P < 0.05$ was considered significant. All calculations represented were determined using SAS version 9.4.

RESULTS

The demographic characteristics of the POISE trial cohort are reported in the original publication[12]. In summary, average (± SD) age was 56 ± 10 years, 91% of patients were female, and 94% Caucasian. The average age at time of PBC diagnosis was 47 ± 11 years, with 93% of patients receiving UDCA for >12 months prior to the beginning of the trial, with a mean daily dose of 16 ± 5 mg/kg. All three patient groups were generally well balanced, as shown in **Table 1**. As reported by *Nevens et al.*[12], there was a statistically significant reduction of the least squares mean values of ALP, ALT, and AST, both in the OCA 5- 10-mg and the OCA 10-mg dose groups compared to the placebo group after 12 months of OCA treatment (**Table 2**). There was a statistically significant difference in mean total bilirubin level in

Table 1. Baseline Demographics

	Placebo ± UDCA (n=73)	OCA 5-10 mg ± UDCA (n=70)	OCA 10 mg ± UDCA (n=73)
Age, years	56 ± 10	56 ± 11	56 ± 11
Female, n (%)	68 (93)	65 (93)	63 (86)
Caucasian, n (%)	66 (90)	67 (96)	70 (96)
Weight, kg	70 ± 13	68 ± 13	71 ± 15
BMI, kg/m^2	26 ± 4	26 ± 5	26 ± 5
UDCA use, n (%)	68 (93)	65 (93)	67 (92)
Daily UDCA dose, mg/kg	15 ± 4	17 ± 5	16 ± 5
Duration PBC, years	8 ± 5	8 ± 6	9 ± 7

Data are Mean ± SD where applicable

Abbreviations: UDCA, ursodeoxycholic acid; OCA, obeticholic acid.

both treatment groups compared to the placebo group after 12 months of treatment. No statistical differences were found in the albumin and platelet count between the three arms.

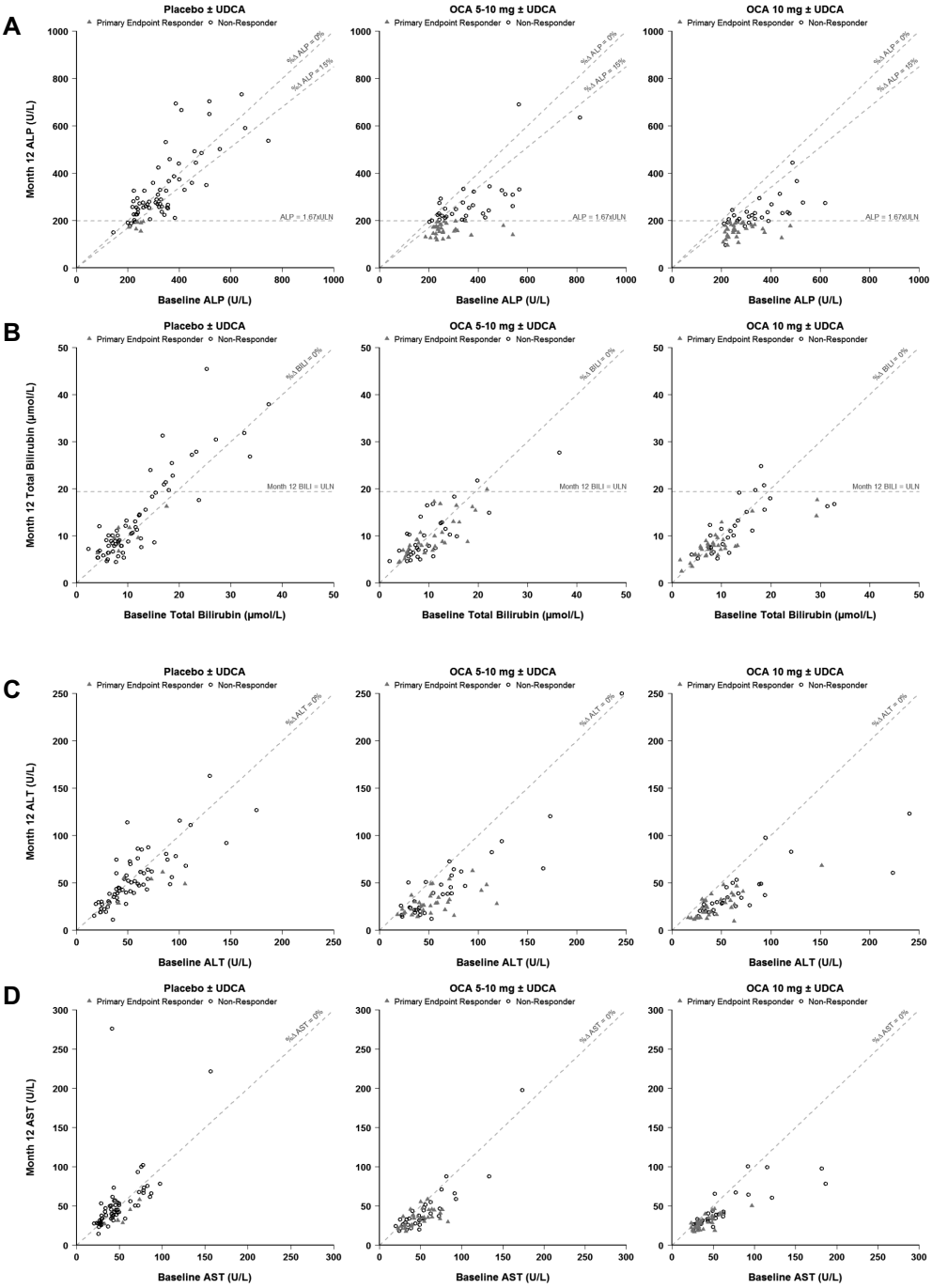
Scatter plots showing changes in the laboratory measurements (included in the scores) after 12 months of treatment and their relationship with the primary composite endpoint (POISE criteria) used in the trial are shown in **Figure 1**. Nearly all OCA-treated patients had a biochemical improvement in ALP, AST, and ALT, including those who did not meet the trial response criteria. No significant differences between OCA and placebo were observed in change from baseline for albumin or platelet count. Baseline and 12-month POISE data were used to calculate the GLOBE score and UK-PBC risk score. Complete biochemical data at 12 months were available for 68, 60, and 59 patients in the placebo, OCA 5-10-mg, and OCA 10-mg treatment groups, respectively. After 12 months of treatment with OCA 6 UDCA, both scores showed reductions in median risk. Assessment of the change in 5-, 10-, and 15-year event risk associated with OCA is shown in **Figure 2**. The comparisons between OCA and placebo arms on the reduction of event risk achieved statistical significance for both OCA dosages across all time points ($P < 0.0001$). Both models predicted improvements in long-term (liver-related and all-cause) risk of mortality or LT after OCA treatment. Furthermore, both models predicted increased risk over time in patients receiving placebo for 1 year, despite 93% of patients receiving concomitant UDCA, the current standard of care for PBC. While both scores showed improvements in projected risk reductions for the OCA treatment groups, the GLOBE score tended to indicate greater worsening in projected risk after 1 year of the placebo group compared with risk predicted by the UK-PBC risk score. Using the GLOBE score, the differences in relative risk reduction from baseline in LT or all-cause mortality after 5, 10, and 15 years between OCA 5-10 mg and placebo were 26.9%, 23.5%, and 20.2%, respectively (**Table 3**). Comparing the difference between OCA 10 mg and placebo, the relative risk reductions were 29.6%, 25.8%, and 22.0%, respectively. Applying the UK-PBC risk score, the differences in relative risk reductions from baseline between OCA 5- 10 mg and placebo of LT or liver-related death after 5, 10, and 15 years were 33.7%, 32.2%, and 30.6%, respectively. The differences in relative risk reduction between OCA 10 mg and placebo were 39.1%, 37.2%, and 35.6%, respectively. Previous studies have demonstrated that patients who are diagnosed with PBC at younger ages may be less likely to respond to UDCA therapy[2]. The change in risk following OCA treatment in both scores was assessed above and below the median age of diagnosis (48 years) in POISE (**Supporting Figure S1**). Both subgroups showed a change in risk consistent with the total POISE cohort.

Patients treated with OCA and diagnosed before the age of 48 years showed a significant reduction ($P < 0.01$) in risk across all estimated time points with the GLOBE score and UK-PBC risk score in contrast to an increase in risk in placebo-treated patients (**Supporting Figure S1A,B**). Similarly, patients diagnosed after the age of 48 or older had significant reductions in projected risk with OCA treatment ($P < 0.01$) with both scores compared to an increase

Table 2. Laboratory Measures at Baseline and 12 Months

	Placebo ± UDCA (n=73)	OCA 5-10 mg ± UDCA (n=70)	OCA 10 mg ± UDCA (n=73)
ALP (U/L)			
Baseline	327.5 (115.0)	325.9 (116.2)	316.34 (103.9)
12 Months	321.3 (142.9)	219.5 (99.8)	192.3 (61.3)
Change from Baseline	-14.4 (14.7)	-112.5 (14.4)***	-129.9 (14.6)***
AST (U/L)			
Baseline	48.8 (22.4)	52.3 (25.3)	50.5 (31.1)
12 Months	51.6 (39.0)	39.5 (25.1)	36.4 (19.2)
Change from Baseline	1.0 (4.2)	-13.0 (4.2)**	-15.0 (4.3)***
ALT (U/L)			
Baseline	56.0 (30.3)	61.6 (39.0)	56.3 (39.7)
12 Months	52.8 (28.5)	39.0 (33.9)	32.1 (20.6)
Change from Baseline	-5.0 (3.3)	-21.3 (3.3)***	-25.3 (3.4)***
Total Bilirubin (µmol/L)			
Baseline	11.8 (7.2)	10.2 (5.5)	11.3 (6.6)
12 Months	13.2 (8.7)	9.9 (4.8)	9.7 (4.7)
Change from Baseline	2.0 (0.7)	-0.3 (0.7)**	-0.9 (0.7)***
Albumin (g/L)			
Baseline	42.8 (3.1)	43.0 (3.1)	43.7 (2.7)
12 Months	41.8 (3.6)	42.7 (3.5)	43.1 (3.3)
Change from Baseline	-1.2 (0.4)	-0.6 (0.4)	-0.9 (0.4)
Platelets (10^9/L)			
Baseline	223.6 (87.1)	224.8 (79.6)	232.9 (87.8)
12 Months	222.5 (101.6)	225.4 (87.0)	228.5 (78.7)
Change from Baseline	6.5 (8.4)	4.9 (8.2)	2.9 (8.5)
Patient Age			
Baseline	55.5 (10.0)	55.8 (10.5)	56.2 (11.0)
12 Months	56.3 (10.2)	56.6 (10.0)	56.2 (10.2)

Baseline and 12 months are Mean (SD), change from baseline data are LS Mean (SE). **p<0.01, ***p<0.0001. P-value for comparing active treatments to Placebo is obtained using an ANCOVA model with baseline value as a covariate and fixed effects for treatment and randomisation strata factor.
Abbreviations: UDCA, ursodeoxycholic acid; OCA; obeticholic acid; ALP, alkaline phosphatase; AST, asparate aminotransferase; ALT, alanine aminotransferase.


A
Placebo ± UDCA
OCA 5-10 mg ± UDCA
OCA 10 mg ± UDCA
Primary Endpoint Responder
Non-Responder
%Δ ALP = 0%
%Δ ALP = 18%
ALP = 1.67xULN
Month 12 ALP (U/L)
Baseline ALP (U/L)
B
%Δ BILI = 0%
Month 12 BILI = ULN
Month 12 Total Bilirubin (µmol/L)
Baseline Total Bilirubin (µmol/L)
C
%Δ ALT = 0%
Month 12 ALT (U/L)
Baseline ALT (U/L)
D
%Δ AST = 0%
Month 12 AST (U/L)
Baseline AST (U/L)

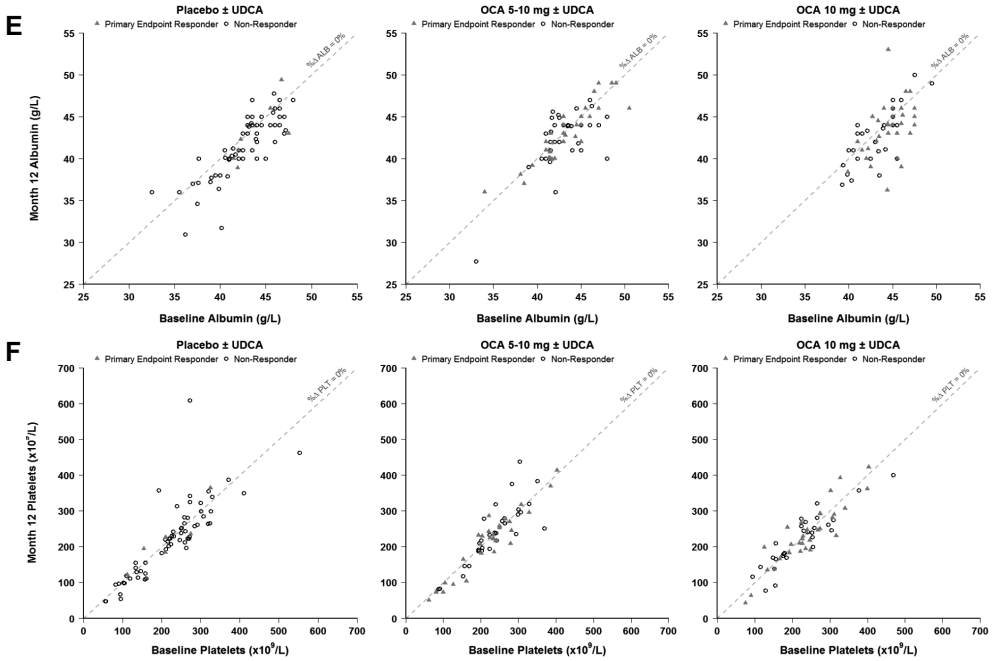


Figure 1. Individual Values in the Biochemical Components of the Globe and UK-PBC Scores. (A) ALP (U/L). (B) Total bilirubin (µmol/L). (C) ALT (U/L). (D) AST (U/L). (E) Albumin (g/L). (F) Platelet Count (x 109/L). All patients are identified as having met the POISE primary response criteria or not. The diagonal line through each plot represents 0% change from baseline; in figure (A) a second diagonal line shown represents a 15% reduction.
Abbreviations: UDCA, ursodeoxycholic acid; OCA; obeticholic acid; ALP, alkaline phosphatase; AST, asparate aminotransferase; ALT, alanine aminotransferase.

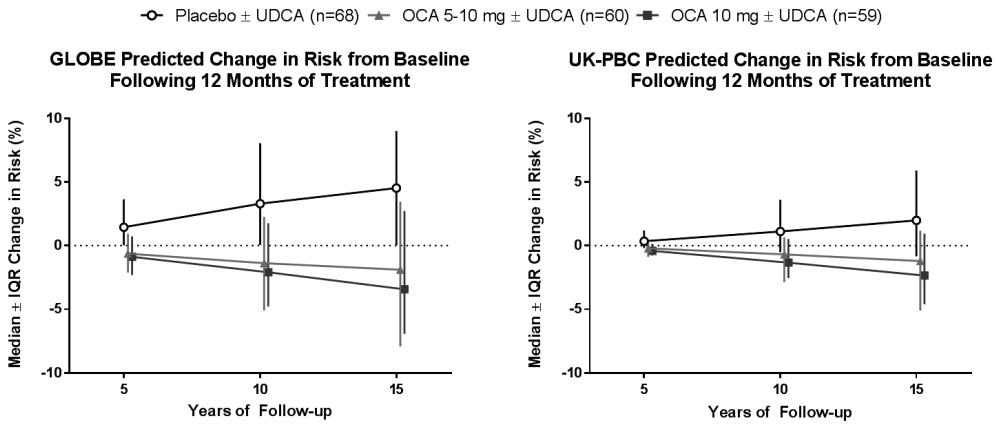


Figure 2. Improvements in Risk with GLOBE Score and UK-PBC Risk Score After 12 Months of OCA Treatment. (A) Predicted median (Q1, Q3) change in risk from baseline with the Globe score. (B) Predicted median (Q1, Q3) change in risk from baseline with the UK-PBC risk score. P<0.0001 for all values in OCA treatment arms in both models. P-value for comparing active treatments to Placebo is obtained using the Rank ANCOVA model with baseline value as a covariate.
Abbreviations: UDCA, ursodeoxycholic acid; OCA; obeticholic acid; IQR, interquartile range.

Table 3. Median Difference in Risk Between Placebo and OCA Treatment Groups After 12 Months of Treatment

	Difference in Estimated Scores		Relative Difference in Estimated Scores†	
	OCA 5-10 mg – Placebo (n=60)	OCA 10 mg – Placebo (n=59)	OCA 5-10 mg – Placebo (n=60)	OCA 10 mg – Placebo (n=59)
GLOBE Score				
5 years	-2.34 (-3.49, -1.30)	-2.56 (-3.65, -1.57)	-26.94 (-38.03, -14.75)	-29.62 (-40.69, -18.82)
10 years	-5.15 (-7.43, -2.92)	-5.67 (-7.72, -3.53)	-23.51 (-33.49, -12.75)	-25.78 (-35.64, -16.60)
15 years	-6.83 (-9.94, -3.81)	-7.38 (-10.19, -4.74)	-20.20 (-28.97, -10.69)	-22.02 (-30.35, -13.85)
UK-PBC Risk Score				
5 years	-0.80 (-1.22, -0.40)	-0.87 (-1.26, -0.53)	-33.65 (-49.64, -17.39)	-39.05 (-54.44, -23.76)
10 years	-2.47 (-3.70, -1.26)	-2.69 (-3.85, -1.68)	-32.18 (-47.87, -16.74)	-37.24 (-52.48, -22.96)
15 years	-4.06 (-6.20, -2.14)	-4.58 (-6.52, -2.83)	-30.64 (-45.81, -15.83)	-35.59 (-49.94, -21.66)

P<0.0001 for all values in OCA treatment arms in both models. All values are Median (95% CI). †Relative differences are based on median differences in % change from baseline between Placebo and OCA.
Abbreviations: UDCA, ursodeoxycholic acid; OCA, obeticholic acid.

in risk with placebo (**Supporting Fig. S1C,D**). Finally, we explored the change in risk for the subgroup of patients classified as inadequate responders to OCA therapy at 12 months. The median change in risk in patients not meeting the POISE primary endpoint after 12 months of OCA, which requires ALP below 1.67 3 ULN with at least a 15% reduction in ALP and total bilirubin at or below ULN, is shown in **Figure 3**. Patients who did not meet the POISE response criteria at month 12 had significant improvements in estimated risk at 5, 10, and 15 years with both scores compared to placebo ($P < 0.01$). We also evaluated the change in risk for patients classified as non-responders by alternative response criteria, including Paris, Rotterdam, Toronto, and Barcelona. For patients who had an inadequate response by these criteria, we report the median change in projected risk following 12 months of treatment as well as median baseline risk for context. Patients classified as non-responders by these criteria showed significant improvements in estimated event risk at 5, 10, and 15 years with both scores after 1 year of OCA treatment compared to placebo in most cases (**Supporting Table S1**).

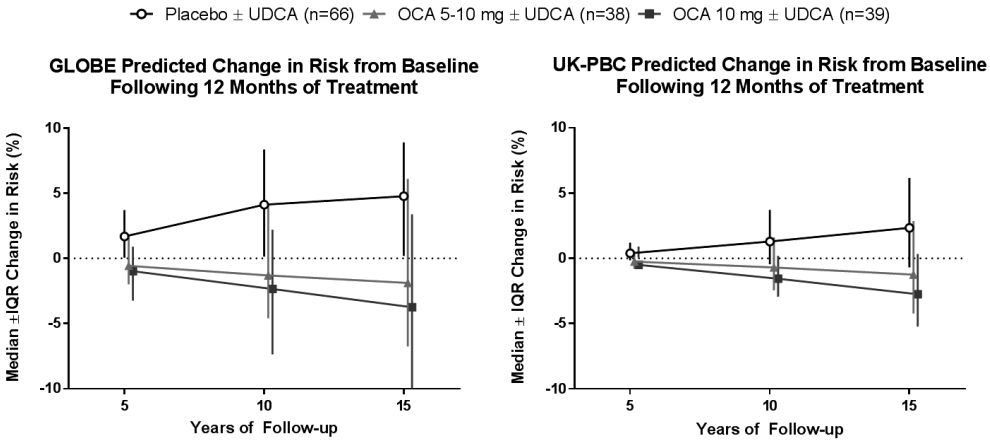


Figure 3. Risk Improvement in OCA "Non-Responders" with GLOBE Score and UK-PBC Risk Score After 12 Months of OCA Therapy. (A) Predicted median (Q1, Q3) change in risk from baseline with the Globe score. (B) Predicted median (Q1, Q3) change in risk from baseline with the UK-PBC risk score. P<0.01 for all values in OCA treatment arms in both models. P-value for comparing active treatments to Placebo is obtained using the Rank ANCOVA model with baseline value as a covariate.
Abbreviations: UDCA, ursodeoxycholic acid; OCA, obeticholic acid; IQR, interquartile range.

DISCUSSION

In this analysis, we quantified the projected risk– benefit of OCA treatment in patients with PBC who inadequately responded or were intolerant to UDCA by applying liver biochemistry data from the phase 3 POISE trial to both the GLOBE score and UK-PBC risk score. Our findings promote the utility of such scoring systems in a clinical trial setting. Moreover, these results shed further light on the nature and scale of benefit from OCA treatment as demonstrated in the POISE trial by *Nevens et al*[12]. Our analysis has two major conclusions. The first is that while the dichotomous POISE trial criteria accurately stratify patients into those at low or high risk of clinical outcomes, the GLOBE score and UK-PBC risk score enable the anticipated survival benefit for PBC patients treated with OCA to be quantified. This is an important step forward in assessing the effectiveness of OCA therapy and other potential new therapies for PBC.

The second conclusion is that the use of dichotomous response criteria in the POISE trial may underestimate therapeutic benefit. This finding is aligned with those presented in the studies in which the GLOBE score and UK-PBC risk score were developed and validated as well as with the results from a Chinese study validating this in a long-term follow-up cohort[18]. These studies all reported that the GLOBE score and UK-PBC risk score were superior in identifying patients with inadequate treatment response when compared to dichotomous response criteria in populations of patients with PBC that were treated with UDCA monotherapy[13,14].

Importantly, we found OCA treatment to be associated with a significant benefit of projected survival, even in patients not reaching the threshold for response by the original POISE criteria and other well-known dichotomous response criteria. This is a result of the inability of the trial criteria to take into account high baseline levels of ALP and/or total bilirubin and subsequent improvements in these markers, which were robust but did not meet the thresholds defined by the primary endpoint. For example, patients with highly elevated ALP levels at baseline (i.e., the highest risk group, in greatest need of improvement) were less likely to meet the dichotomous response criteria even when a substantial reduction of ALP and/or total bilirubin was seen. The therapeutic benefit overlooked by using the dichotomous trial criteria not only implies an underestimation of efficacy but could also impact future treatment options for the most severely affected patients, especially given the current cost–utility analysis of OCA as measured by the incremental cost-effectiveness ratio threshold that may not be applicable to a rare disease[19].

In this analysis, we observed differences in the projected risks between the GLOBE score and the UKPBC risk score. These differences are most likely explained by the different endpoints used in the two scores; the GLOBE score takes into account LT and all-cause mortality while the UK-PBC risk score considers LT and liver-related death. Consequently, as the GLOBE score considers all causes of death, the baseline risk of an endpoint is higher using the GLOBE score. Likewise, the risk of all-cause mortality increases faster over time (with aging) than the risk of liver-related death and therefore shows a steeper trajectory of risk for the GLOBE score. We acknowledge that the two scoring systems were developed to predict adverse outcome in patients taking UDCA. Neither score was validated in patients taking OCA. We believe the scores are applicable in the current context, however, because both were validated in cohorts of patients who had not received treatment with UDCA. Nevertheless, neither score can identify effects of OCA independent of those reflected by changes in liver biochemistry on treatment. In addition, we acknowledge the limitation that both scores depend on endpoints that are surrogates for clinical outcomes. Although these surrogate endpoints for outcome are likely to be accurate, other factors, such as toxicity or other adverse events, should not be overlooked when evaluating risks and benefits of new therapeutic agents. Therefore, the accuracy of predictions in the current analysis await confirmation by the ongoing phase 4 trial of OCA evaluating clinical outcomes in patients with PBC (COBALT; NCT02308111)[20]. This analysis showed a median reduction in the 10-year event risk of 2.1% using the GLOBE score and 1.3% using the UK-PBC risk score after 12 months of treatment with OCA 10 mg compared to a median increase of 3.3% (GLOBE) and 1.1% (UK-PBC) after 12 months of placebo. This represents a difference in relative risk reduction from baseline between OCA 10 mg and placebo of 25.8% with the GLOBE score and 37.2% with the UK-PBC risk score. However, we emphasize that this is a selected trial cohort. In a real-life population with more advanced or aggressive disease, the impact of this new treatment might be different. Importantly, transient elastography is not included in either of

the scores. Despite the prognostic value of transient elastography[21-23], a great advantage of both presented risk scores in our study is that they are able to predict outcomes accurately based on readily available biochemical parameters and without the need for a dedicated instrument and skilled operator required for transient elastography.

In conclusion, we found that 1 year of OCA therapy was projected by both scores to reduce the risk of death and LT in this patient population, including patients not meeting the dichotomous POISE primary endpoint. We believe that the application of the GLOBE score and UK-PBC risk score in clinical practice would be an important step toward individualizing risk prediction in PBC and may eventually replace the use of other dichotomous therapy response criteria in clinical practice.

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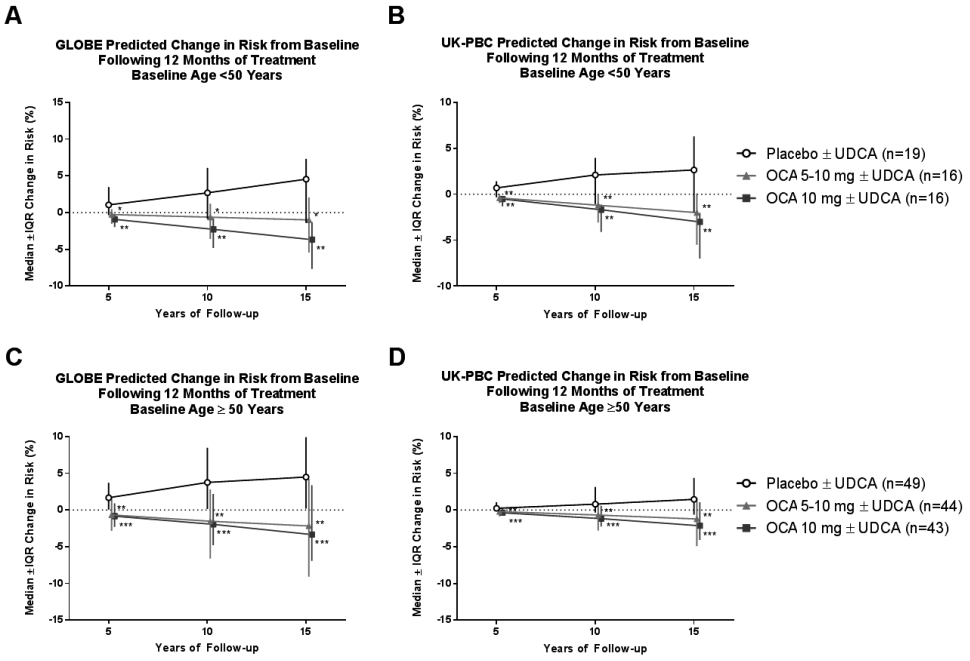
SUPPLEMENTARY DATA



Supplemental Figure 1. Improvements in Risk with GLOBE score and UK-PBC Risk Score After 12 Months of OCA Treatment by Median Age of PBC Diagnosis. (A) Predicted median (Q1, Q3) change in risk from baseline with the *Globe score* in patients diagnosed with PBC before the age of 48 years. **(B)** Predicted median (Q1, Q3) change in risk from baseline with the *UK-PBC risk score* in patients diagnosed with PBC before the age of 48 years. **(C)** Predicted median (Q1, Q3) change in risk from baseline with the *Globe score* in patients diagnosed with PBC at the age of 48 years or later. **(D)** Predicted median (Q1, Q3) change in risk from baseline with the *UK-PBC risk score* in patients diagnosed with PBC at the age of 48 years or later. P<0.01 for all values in OCA treatment arms in both models**.** P-value for comparing active treatments to placebo is obtained using the Rank ANCOVA model with baseline value as a covariate.
Abbreviations: IQR, interquartile range; UDCA, ursodeoxycholic acid; OCA, obeticholic acid.

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CHAPTER 8
TRENDS IN LIVER TRANSPLANTATION FOR PRIMARY BILIARY CHOLANGITIS IN EUROPE OVER THE PAST THREE DECADES

Alimentary Pharmacology & Therapeutics, 2018

ABSTRACT

Background The importance of primary biliary cholangitis (PBC) as indication for liver transplantation (LT) over time is likely influenced by the introduction of therapies, and changes in selection criteria and disease epidemiology.

Aim We aimed to assess time trends in LT for PBC and to evaluate characteristics of the patient population during the past three decades.

Methods Patients undergoing LT from 1986-2015 in centres reporting to the European Liver Transplantation Registry were included. We excluded combined organ transplantations and patients <18 years. Trends were assessed using linear regression models.

Results We included 112,874 patients that underwent LT between 1986-2015, of which 6029 (5.3%) patients had PBC. After an initial increase in the first decade, the annual number of LT for PBC remained stable around 200. The proportion of LT for PBC decreased from 20% in 1986 to 4% in 2015 (p<.001). PBC was the only indication showing a consistent proportional decrease throughout all decades. From the first to the third decade, the age at LT increased from 54 (IQR47-59) to 56 years (IQR48–62) and the proportion of males increased from 11% to 15% (both p<.001). MELD scores increased from 15 (IQR12-19) in 1996-2005, to 17 (IQR13-22) in 2006-2015 (p<.001).

Conclusions In our European-wide study spanning 30 years, we found a proportional decrease in PBC as indication for LT. However, despite treatment with ursodeoxycholic acid and improved disease awareness, the absolute annual number of LTs has stabilized and LT remains indicated for a subgroup of PBC patients.

INTRODUCTION

Primary biliary cholangitis (formerly called primary biliary cirrhosis, PBC) is a chronic cholestatic liver disease, characterized by progressive intrahepatic bile duct destruction which may eventually lead to fibrosis, cirrhosis, and death[1].In the 20 years following the first human liver transplantation in 1963, PBC was the leading indication for liver transplantation in Europe, accounting for 30%-50% of all liver transplantations[2].However, despite increasing disease prevalence[3,4], PBC is no longer a leading indication for liver transplantation. The recent change in nomenclature is figurative for the changed prognosis of patients over the past decades[5].

Today, patients are usually diagnosed with PBC at an early stage, which allows for timely onset of therapy, often resulting in improvement of biochemical parameters and prognosis[6–12]. However, patients who respond incompletely still have a significantly impaired prognosis compared to an age- and sex-matched general population[13,14]. In case of liver failure, liver transplantation remains the only therapeutic option to prevent premature death. However, changes in selection criteria for liver transplantation and in the epidemiology, as well as the introduction of ursodeoxycholic acid (UDCA) as an effective treatment, may have impacted the relative importance of PBC as indication for transplantation.

Previous European studies that have assessed trends in liver transplantation for PBC reported a decrease in transplantations. However, these studies were either single center or single-country studies, often covered relatively short study periods, or are now outdated[15–17]. A long-term European-wide study is currently lacking, as is data on possible changes over time in the characteristics of the subgroup of patients with PBC who still require liver transplantation. Thus, the primary aim of this study was to assess the time trends in the number of liver transplantations for PBC across Europe over the past three decades, both in absolute and proportional measures. Secondly, we aimed to evaluate the potential changes in characteristics of patients with PBC undergoing liver transplantation during this time period.

MATERIALS AND METHODS

Study design

Patient data were obtained through the European Liver Transplantation Registry (ELTR). ELTR data are available to all the members of the European Liver and Intestine Transplant Association (ELITA) for research purposes, once the study protocol is approved. All patients transplanted in ELTR-associated centers from 1 January 1986 until 31 December 2015, were assessed. Patients who underwent combined organ transplantation and patients aged under 18 years at the time of liver transplantation were excluded. In order to more thoroughly assess patients' characteristics, all patients with PBC who were listed for liver transplantation at any of the three liver transplantation centers in the Netherlands during the study period were included for in-depth analyses. This study was conducted in accordance with the principles of the Declaration of Helsinki. The protocol was approved by ELITA and the Dutch Organ Transplant Registry. 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.

Data collection

Data collected for our primary analyses included date of birth, gender, date of listing for liver transplantation, date of liver transplantation, and the biochemical parameters used to calculate the model for end-stage liver disease (MELD) score at the time of transplantation.18 Indications for liver transplantation by primary and secondary etiologies were classified using the ELTR etiology codes. Patients with PBC were identified using these etiology codes, including patients with PBC-autoimmune hepatitis (PBC-AIH) overlap syndrome (defined as interface hepatitis on liver histology combined with alanine aminotransferase (ALT) ≥5× upper limit of normal or IgG ≥2×)19 and patients with an additional diagnosis of hepatocellular carcinoma. For our in-depth analyses, all patients with PBC listed for liver transplantation during the study period in the Netherlands were identified using the Dutch Organ Transplant Registry (NOTR) and the three local liver transplantation center registrations. For these patients, the following additional data were extracted from medical records: date of PBC diagnosis, date of initiation of UDCA treatment when applicable, secondary indication for liver transplantation and biochemical parameters including total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), albumin, creatinine, and INR at time of diagnosis, at time of initiation with UDCA treatment, at 1 year after treatment initiation, at time of listing for liver transplantation, and at time of liver transplantation. These measures were used to calculate the Barcelona, Paris I, and GLOBE response criteria for patients 1 year after the initial start of UDCA treatment[14,20,21]. The secondary indication for liver transplantation was classified as either liver failure, an additional diagnosis of hepatocellular carcinoma, or quality of life (QOL) (defined as therapy-refractory fatigue or pruritus after following the EASL guidelines for management of cholestatic liver diseases or a mean score of ≥4 in the

fatigue or pruritus domain of the PBC-40)[22]. The biochemical disease stage was determined based on serum albumin and bilirubin concentrations according to the Rotterdam criteria, classifying PBC into early (normal total bilirubin and normal albumin), moderately advanced (abnormal total bilirubin or abnormal albumin), or advanced disease (abnormal total bilirubin and abnormal albumin).10 UDCA treatment status was classified as "yes" when patients were treated with UDCA at time of listing, independent of treatment duration, dosage, and/or combination with other treatment.

Calculations

MELD scores provided in the registry data were used when available, also when this concerned patients with MELD exception points. If no MELD score was declared, we calculated it based on laboratory values using the following formula: 0.957 x Natural logarithm (ln) of (creatinine in mg/dL) + 0.378 x ln (bilirubin in mg/dL) + 1.120 x ln (INR) + 0.643.18 For our analyses, we preferred declared MELD scores over laboratory MELD scores when available, since laboratory MELD scores do not take into account possible MELD score exception points. To exclude erroneous data, for all biochemical parameters included in the MELD score, clinically feasible minimum and maximum values were defined based on clinical expertise. Values exceeding these ranges were excluded from analyses and considered missing. The ranges were defined as follows: serum creatinine 0.01-11.3 mg/dL (20-1000 µmol/L), serum bilirubin 0.06-58 mg/dL (1-1000 µmol/L), INR 0.5-10, and albumin 10-60 g/L. The MELD score was considered to range from a minimum of 6 to a maximum of 40[23].

Statistical analyses

Linear regression least-square models were used to assess trends over time for our primary endpoints. For normally distributed continuous variables, the one-way ANOVA was used for the comparison of more than two groups. In case of skewed distribution of continuous variables, the Mann-Whitney U test was used for the comparison of two groups, and the Kruskal-Wallis test for more than two groups. For categorical variables, differences between the three decades were compared using the chi-squared test. For comparative purposes, the study period was divided into three groups according to the date of transplantation. Every group represents the time span of one decade (1 January 1986 - 31 December 1995; 1 January 1996 - 31 December 2005; 1 January 2006 - 31 December 2015). Statistical analyses were performed using SPSS version 21. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Study population

Between 1 January 1986 and 31 December 2015, 128 802 patients underwent liver transplantation in 166 European Liver Transplantation Registry centres. We excluded 15 928 patients because of combined organ transplantation or age <18 years at time of liver transplantation. Thus, 112 874 patients were included in our study population. Of this total, 6029 (5.3%) were transplanted for PBC. In comparison, 26 861 (23.8%) were transplanted for viral hepatitis, 23 207 (20.6%) for alcoholic cirrhosis, 20 047 (17.8%) for cancers, and 9226 (8.2%) for autoimmune diseases of the liver other than PBC. Patient characteristics for those transplanted for PBC are presented in **Table 1**.

Primary Indications for liver transplantation — changes in absolute and proportional numbers

In 1986, 283 liver transplantations were performed, as compared to 5646 in 2015. The number of transplantations increased by 184 per year (95% CI 183-184, $P < 0.001$) (**Figure 1A**). The absolute annual number of transplantations for PBC peaked to 279 in 1994. Thereafter, the annual transplant rate for PBC decreased to an average of 200 in the last decade (Figure 1B). These changes correspond with an average annual increase of 21.5 (95% CI 21.3-21.7; $P < 0.001$) transplantations in the first decade, followed by a decrease of −1.9 (95% CI −2.1 to −1.7; $P < 0.001$) in the second decade, and a marginal annual decrease of −0.3 (95% CI −0.5 to −0.1; $P = 0.002$) in the last decade. The proportion of patients undergoing liver transplantation for PBC as compared to other indications decreased from 20.3% in 1986% to 3.7% in 2015 (**Figure 2A**). The greatest decrease was in the first decade, with an annual

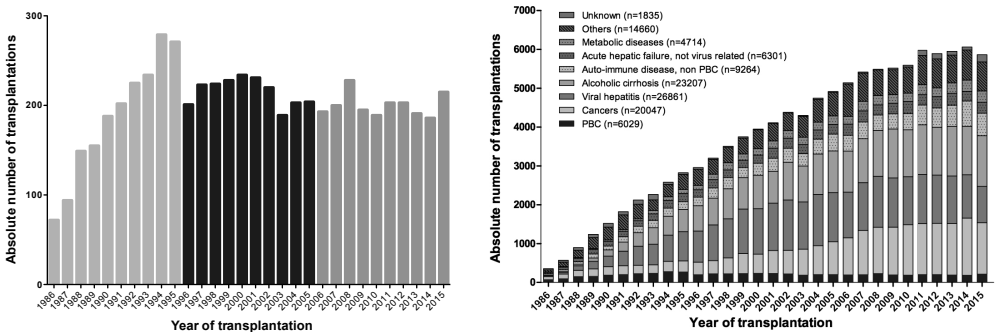

Figure 1. Annual absolute number of liver transplantations in ELTR centres from 1986-2016 for (A) all primary disease etiologies, and (B) PBC.

Table 1. Characteristics of patients transplanted for PBC in ELTR centres from 1986-2016.

	Overall	Decade of Liver Transplantation			
	1986-2016	**1986-1996**	**1996-2006**	**2006-2016**	**p – value**
	N = 6029	N = 1869	N = 2157	N = 2003	
Age [a]					
Overall	55.2 (48.1-61.2)	53.9 (47.3-59.4)	55.7 (49.0-61.4)	56.1 (48.4-62.4)	<.001
Female	55.1 (48.1-61.1)	53.6 (47.1-59.1)	55.6 (49.0-61.4)	55.9 (48.6-62.3)	<.001
Male	55.2 (48.1-61.2)	55.6 (47.3-59.4)	57.0 (48.1-62.1)	56.9 (48.4-62.4)	.616
Gender (%)					
Female	5267 (87.4)	1664 (89.0)	1904 (88.3)	1699 (84.8)	.532
Male	761 (12.6)	205 (11.0)	253 (11.7)	303 (15.1)	
PBC (%)					
PBC	5907 (98)	1866 (99.8)	1904 (88.3)	1699 (84.8)	<.001
PBC – AIH	38 (0.6)	0 (0.0)	10 (0.5)	28 (1.4)	
PBC – HCC	84 (1.4)	3 (0.2)	24 (1.1)	57 (2.8)	
MELD score [a]	16.2 (12.7-20.9)	17.0 (13.8-20.5)	15.3 (12.3-19.1)	16.8 (12.8-21.7)	<.001
Creatinine (mg/dL) [a]	0.9 (0.7-1.1)	1.0 (0.81-1.26)	0.9 (0.8-1.1)	0.80 (0.6-1.1)	<.001
Bilirubin (mg/dL) [a]	5.9 (2.7-11.8)	7.2 (3.5-13.2)	5.4 (2.7-10.3)	6.0 (2.6-12.9)	.001
INR [a]	1.3 (1.1-1.5)	1.2 (1.1-1.5)	1.2 (1.1-1.4)	1.3 (1.1-1.6)	<.001
Albumin (g/L) [b]	31.0 (27.0-36.0)	30.0 (26.0-35.0)	31.0 (27.0-36.0)	31.0 (26.6-36.0)	.137

a. Shown as median (IQR)
b. Shown as mean ± sd
Missing values: INR n=9 (6%), MELD score n=9 (6%).
Abbreviations: PBC, primary biliary cholangitis; AIH, auto immune hepatitis; HCC, hepatocellular carcinoma; QOL, quality of life; MELD, model for end-stage liver disease; INR, internationalized normal ratio.

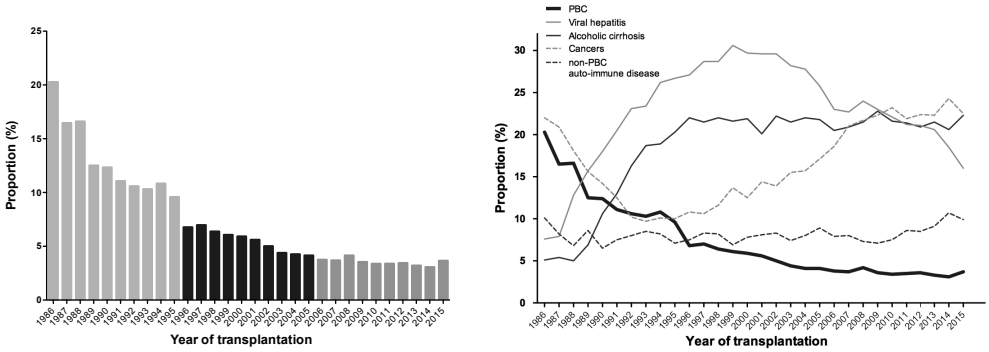

Figure 2. Proportion of liver transplantations in ELTR centres from 1986 to 2016 for (A) PBC and (B) PBC and other leading indications.

proportional decrease of 0.9% (95% CI −0.92 to −0.88; $P < 0.001$). Thereafter, the proportion of transplantations for PBC annually decreased with 0.3% (95% CI −0.343 to −0.337; $P < 0.001$) in the second decade and 0.1% (95% CI −0.064 to −0.057; $P < 0.001$) in the third decade. No other leading disease aetiology showed a persistent significant proportional decrease over all three decades **(Figure 2B**).

Age

The overall median age at time of transplantation was 55 years (interquartile range [IQR] 48-61) for patients with PBC, and 53 years (IQR 44-59) for non-PBC patients ($P<.001$). For PBC patients, the median age at time of transplantation increased significantly from 54 (IQR 47-59) in the first decade to 56 (IQR 49-61) in the second decade ($P < 0.001$), while no significant age difference was found thereafter ($P = 0.255$) (**Figure 3**). Furthermore, a change in distribution of patients' age at time of transplantation was found (**Figure 4**). The proportion of patients aged >60 increased from 23% in the first decade to 35% in the last decade ($P > 0.001$), whereas the proportion of patients between 40-49 and 50-59 years decreased from 27% and 42% in the first decade, to 21% and 36% respectively in the last decade (both $P < 0.001$).

Gender

A total of 761 (12.6%) male and 5267 (87.4%) female patients with PBC were transplanted, corresponding to a male to female ratio of 1:6.9. For non-PBC aetiologies, 74 484 (69.7%) males and 32 348 (30.3%) females underwent transplantation, corresponding to a female to male ratio of 1:0.43. In PBC patients, the proportion of transplantations for males increased significantly from 11.0% in the first decade to 15.1% in the third decade ($P < 0.001$) (Figure 5). In the first decade, males were significantly older than females with a median age of 56 years (IQR 49-61) and 55 years (IQR 48-60) respectively ($P < 0.05$), but no differences were found in the second ($P = 0.755$) and third decades ($P = 0.695$) (**Figure S1A**).

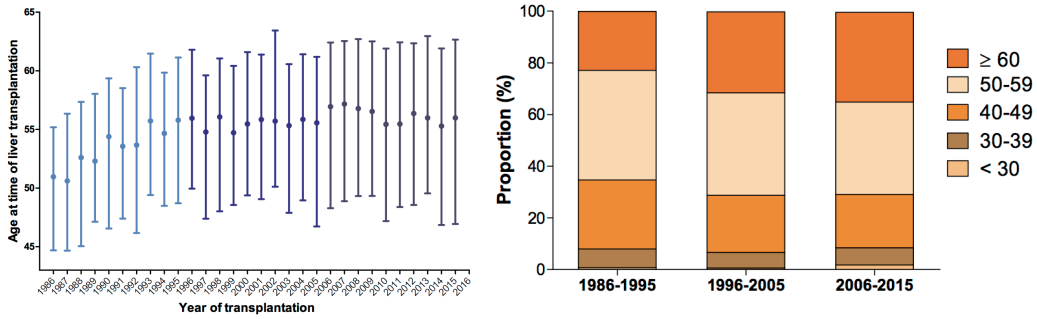

Figure 3. A) Age (median, interquartile range) at time of liver transplantation for PBC in Europe from 1986 to 2016, B) Proportional age distribution for transplanted PBC patients in Europe from 1986-2016.

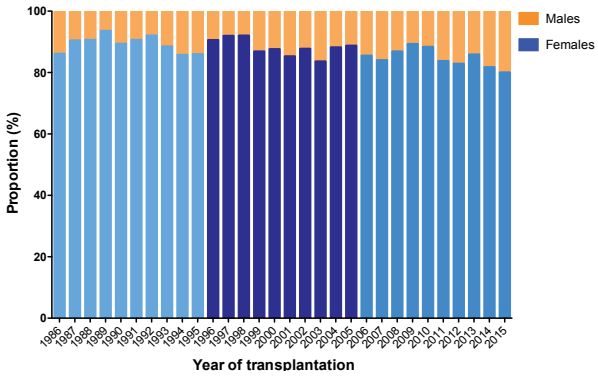

Figure 4. Percentage of male versus female patients transplanted for PBC in Europe from 1986 to 2016.

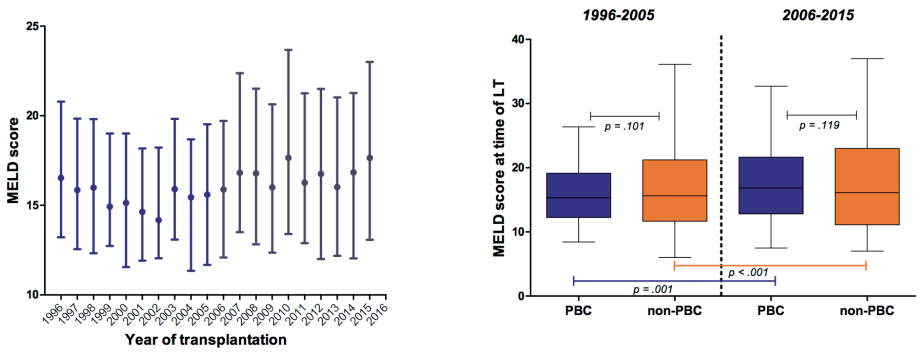

Figure 5. a) MELD scores at time of LT for patients with PBC transplanted in Europe from 1996-2015; b) MELD scores of patients transplanted for PBC versus non-PBC aetiologies in 1996-2005 versus 2006-2015. *MELD scores are reported as median with their interquartile ranges. As a result of a lack of biochemical data from the first decade of the study period, data is shown for 1996-2015. In this period, MELD scores were available for 2,749 (46%) of patients with PBC and 51,410 (48%) of non-PBC patients.*
Abbreviations: LT, liver transplantation; MELD, model for end-stage liver disease.

MELD scores

In patients with PBC, the median MELD score at time of transplantation increased from 15.3 (IQR 12.2-19.2) in the second decade to 16.8 (IQR 12.8-21.6) in the last decade ($P < 0.001$). An increase in MELD score between these decades was also found in non-PBC liver transplantations, in which the median MELD score increased from 15.6 (IQR 11.7-21.2) to 16.1 (IQR 11.1-23.0) ($P < 0.001$) (**Figure 5**). There were no significant differences in MELD scores between PBC and non-PBC patients in either the second or the third decade (P = 0.101 and P = 0.119 respectively). In PBC patients, no significant difference in MELD score was found between females (16.1, IQR 12.5-20.8) and males (16.9, IQR 13.0-21.0) (P = 0.160).

CHARACTERIZATION OF NATIONWIDE COHORT

Mortality on the waiting list

From 1 January 1986 to 31 December 2015, 184 patients with PBC were placed on the waiting list for transplantation in the Netherlands. Of this total, one patient (0.5%) was alive without transplantation at the end of follow up. Twenty-nine (15.8%) patients died on the waiting list or were removed from the waiting list due to clinical deterioration. Eight of these events occurred between 1986 and 1995, eight between 1996 and 2005, and 13 between 2006 and 2015, corresponding to a waitlist mortality of 12%, 16%, and 33% in the consecutive decades. In addition, three (1.6%) patients were removed at personal request or due to improved condition. The remaining 151 (82.1%) were transplanted.

Study population

Characteristics and biochemistry of the 151 patients who were transplanted for PBC in the Netherlands are presented in **Table S1**. The median age at the time of transplantation was 54 years (IQR 48-59). Transplanted patients were diagnosed with PBC at a median age of 46 (IQR 40-51), and listed at a median age of 53 (IQR 47-59). Males were significantly older than females at the time of transplantation, with a median age of 58 (IQR 53-64) as compared to 53 (IQR 49-58) (P = 0.006), while the MELD score at the time of liver transplantation did not differ between the sexes (P = 0.838).

Primary indications for liver transplantation: Absolute changes over time

The absolute number of transplantations for PBC nearly halved over time, from 65 in the first decade to 36 in the third decade (P < 0.001) (Figure S2). Of the 151 patients transplanted for PBC, 129 (85.4%) were listed for the primary indication PBC alone, 16 (10.6%) patients had a PBC-AIH overlap syndrome, and six (4.0%) patients had an additional diagnosis of hepatocellular carcinoma. The proportion of patients with PBC transplanted due to poor

quality of life significantly increased from 2% to 12% to 17% in the three consecutive decades (P = 0.004).

UDCA treatment and response for all patients with PBC

A total of 102 (67.5%) transplanted patients were treated with UDCA at the time of listing. The proportion of patients who were treated with UDCA increased significantly over time, from 37% in the first, to 92% in the last decade (P < 0.001). **Table 2** shows the biochemical response for these UDCA-treated patients according to the Barcelona, Paris I, and GLOBE response criteria.14,20,24,25 In this cohort of transplanted patients with PBC, 6-47% of patients showed a biochemical response to treatment, depending on the response criteria. The Paris I criteria identified the lowest percentage of complete biochemical responders after 12 months of UDCA treatment (6%). When classification by different response criteria was compared, this proportion was significantly lower than the percentage of complete responders as classified by the Barcelona criteria (30%) (P = 0.008) and by the GLOBE criteria (28%) (P = 0.004).

Table 2. Biochemical response to 12 months of UDCA therapy in patients that underwent LT in the Netherlands between 1986-2016.

	Overall	*Decade of Liver Transplantation*		
	1986-2016	**1986-1996**	**1996-2006**	**2006-2016**
	N = 102	*N = 28*	*N = 41*	*N = 33*
Barcelona criteria				
Responder, n(%)	16 (30)	3 (20)	10 (40)	3 (21)
Incomplete responder, n(%)	38 (70)	12 (8)	15 (60)	11 (79)
Missing, n(%)	48 (47)	13 (46)	16 (39)	19 (58)
Paris I criteria				
Responder, n(%)	4 (6)	1 (5)	1 (4)	2 (10)
Incomplete responder, n(%)	61 (94)	19 (95)	26 (96)	18 (90)
Missing, n(%)	37 (36)	8 (29)	14 (34)	15 (46)
GLOBE criteria				
Responder, n(%)	16 (28)	0 (0)	9 (39)	7 (39)
Incomplete responder, n(%)	41 (72)	16 (100)	14 (61)	11 (61)
Missing, n(%)	45 (44)	12 (43)	18 (44)	15 (46)

DISCUSSION

This study represents the largest European-wide study on time trends in liver transplantations for PBC to date, covering the past 30 years. The percentage of transplantations for PBC as compared to other etiologies decreased to less than one-fifth of its original proportion of 20%. In contrast, the absolute number of transplantations for PBC has remained virtually stable over the last 10 years. Characteristics of patients undergoing transplantation for PBC have changed over time, whereby they are now older, have higher MELD scores, and are more likely to be male than 30 years ago. However, differences over time were quantitatively small.

The current study is the first to demonstrate a stable annual absolute number of transplantations for PBC over the past 10 years in Europe. This result confirms that today, in a minority of patients, we are still unable to prevent liver failure which underlines the necessity of additional therapeutic options for this group. A study by *Lee et al.* that assessed transplantations trends for PBC in the United States showed an absolute annual decrease without reaching a steady state, but their study period only covered 11 years and ended in 2006[13]. Our finding seems in contrast to the recent study by *Webb et al.* reporting a decrease in the United Kingdom and the United States up until 2014. However, a true comparison is difficult, since the latter study only reported listings for transplantation and not actual liver transplantation, and measured numbers as a ratio against the total (increasing) general population. However, the steady absolute number of transplantations for PBC is also discordant with an overall increase in overall numbers of annual transplantations and with the increasing prevalence of PBC over the past decades[3,4]. Therefore, the stable absolute number of transplantations for PBC that we report could possibly be interpreted as a relative decrease.

Although we found an annual number of approximately 200 transplantations for PBC over the past 10 years the true number of PBC patients in need of liver transplantation may be higher. First, 16% of all listed patients with PBC in our secondary analysis in the Dutch cohort died while on the waiting list for transplantation or were removed due to deteriorating clinical condition. This result is in line with a recent study showing a waiting list mortality of 12% for PBC patients, which was higher than for most other aetiologies[26]. Second, as compared to the number of Dutch patients who underwent transplantation for PBC reported in the ELTR database, we identified an additional 10% of patients after extensive review of medical records of all listed patients in the entire Dutch cohort. Evidently, not all transplanted patients with PBC had been reported as such, and were possibly labelled with more general etiologies lacking further specification, such as cirrhosis or autoimmune disorders. Even more so, a minority of patients might have been incorrectly diagnosed when PBC was not recognized by treating physicians as the underlying cause of cirrhosis. Furthermore, within the top five primary indications for transplantation, PBC was the only

one that showed a constant proportional decrease over three decades. We speculate that several factors may have contributed to this decrease. First is the possible influence of UDCA treatment. Recently, the Global PBC Study Group reported a strong association between UDCA treatment and improved transplant-free survival[11]. The number of transplantations for PBC peaked several years after UDCA was introduced in the early nineties, with a subsequent much more stable number of transplantations. Second, the introduction of urgency-based allocation may have influenced the proportional decrease in transplantations for PBC. Since MELD-based allocation in 2006, patients' time on the waitlist is no longer a factor in allocation, possibly impeding the chance of receiving a transplantation for patients with a relatively slowly progressing disease such as PBC[1].

We also identified several changes in the characteristics of patients undergoing transplantation for PBC over the past three decades. We found an increase in age at time of transplantation. This was in line with the results from a long-term study from Birmingham and a recent larger study covering both the UK and the USA[16,27]. Most noticeable, however, was our finding of an increasing proportion of patients with PBC transplanted at age >60 years. This may be related to an overall improved medical care and physical condition, permitting transplantation at higher ages. The influence of possible changes in environmental triggers involved in the development of PBC should also be considered, especially since a recent large study showed that the age at PBC diagnosis has markedly increased over the years[28]. However, with a median age at diagnosis of 46 years in patients with PBC listed for transplantation within our in-depth population, this population undergoing liver transplantation for PBC was much younger at diagnosis than the large overall cohorts reported by the Global PBC Study Group (55 years) and the UK-PBC Study Group (55 years)[21,29]. This suggests that young patients may be more likely to develop end-stage disease requiring liver transplantation, as has previously been reported[30]. Furthermore, we found that an increasing number of males were transplanted for PBC over time, which was also reported in the United States[27]. The increasing male to female ratio reported in epidemiological studies could contribute to these findings, as well as data that suggest males are less likely to respond well to treatment with UDCA[30,31]. The MELD score at time of transplantation was slightly but significantly higher after 2006, as compared to the period between 1996 and 2005. This might be explained by the introduction of MELD-based allocation in 2006. This increase was found for both PBC and non-PBC patients, although more pronounced in patients with PBC. We identified no differences in MELD scores between PBC and non-PBC patients.

In our in-depth analysis of the Dutch cohort, the patients' characteristics were comparable to the European-wide cohort. Notably, we found that the proportion of patients with PBC who underwent transplantation for poor quality of life increased from 2% before 1995 to 17% in the last decade. Furthermore, we found that despite international guidelines recommending that all patients with PBC be treated with UDCA[32,33], 8% were not treated

with UDCA during the last decade. Although we were unable to validate these findings in the European data, these factors might also have impacted the remaining number of liver transplantations for PBC over the last decade.

To the best of our knowledge, our study was the first to assess time trends in liver transplantation for PBC throughout Europe over a sizeable period of 30 years, during which UDCA was introduced as the standard of care. We were able to include all liver transplantations reported to the ELTR, resulting in a large population of 6029 transplanted PBC patients. The additional nationwide in-depth analysis over the same study period enabled us to characterize the population in need for liver transplantation more extensively over time. However, some limitations should be taken into account. Biochemical data were incomplete in both the ELTR data and the Dutch in-depth analysis. However, due to the large population and the similar patterns found in both analyses, we believe this has not introduced an important bias. Second, our in-depth analyses are based on data of Dutch patients only and the extent to which findings can be generalized is uncertain. Nevertheless, since patient characteristics and MELD scores at transplantation were comparable to the PBC population in the ELTR database and similar transplantation patterns over time were observed, the results of our nationwide in-depth analyses may well be representative of the European PBC population in need of liver transplantation.

In conclusion, we found that, despite a relative decrease, the absolute number of transplantations for PBC has reached a steady state. Still, over 200 European patients with PBC undergo liver transplantation annually. These patients are slightly older, have higher MELD scores, and are more likely to be male than 30 years ago. Effective second-line therapies may further reduce the need for liver transplantation in patients with PBC in the future.

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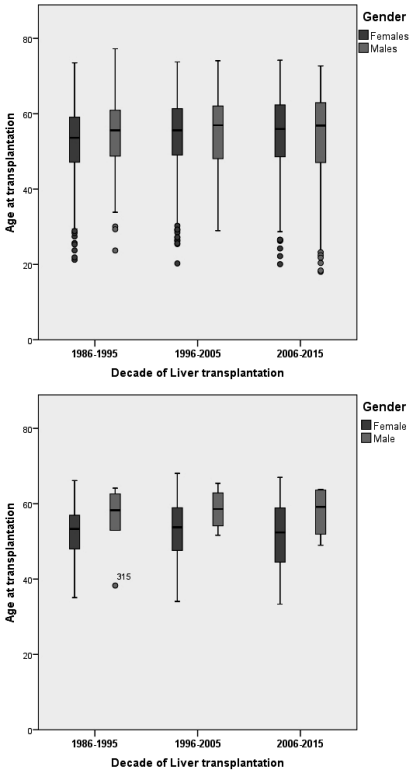
SUPPLEMENTARY DATA



Supplementary Figure S1. Age at time of liver transplantation per decade stratified by gender for patients with PBC transplanted in (A) Europe, and (B) the Netherlands from 1986-2016.

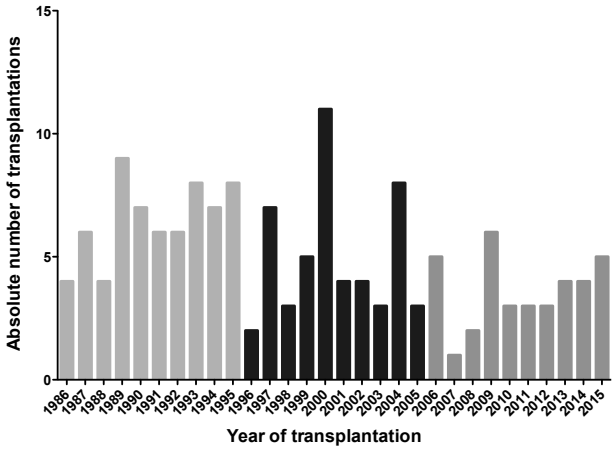


Figure S2. Annual absolute number of liver transplantations for PBC patients in the Netherlands from 1986-2016.

Supplementary Table S1. Characteristics of patients transplanted for PBC in the Netherlands centres from 1986-2016.

	Overall	*Decade of Liver Transplantation*			
	1986-2016	**1986-1995**	**1996-2005**	**2006-2015**	**p - value**
	N = 151	*N = 65*	*N = 50*	*N = 36*	
Age at LT [a]					
Overall	53.6 (47.8-58.6)	53.4 (48.0-57.8)	54.3 (48.2-60.7)	52.9 (44.6-60.4)	.442
Female	53.1 (46.8-57.6)	53.3 (48.0-57.1)	53.8 (47.5-59.0)	52.4 (44.5-59.6)	.596
Male	58.3 (53.4-63.5)	58.3 (49.3-63.0)	58.6 (53.9-63.7)	59.2 (50.5-63.7)	.921
Gender (%)					
Female	133 (88.1)	59 (90.8)	42 (84.0)	32 (88.9)	.532
Male	18 (11.9)	6 (9.2)	8 (16.0)	4 (11.1)	
PBC (%)					<.001
PBC	129 (84.9)	64 (98.5)	40 (80.0)	25 (69.4)	
PBC – AIH	16 (10.5)	1 (1.5)	8 (16.0)	7 (19.4)	
PBC – HCC	6 (4.6)	0 (0.0)	2 (4.0)	4 (11.1)	
Sub indication (%)					.004
Liver Failure	135 (89.4)	64 (98.5)	43 (86.0)	28 (77.8)	
HCC	3 (2.0)	0 (0.0)	1 (2.0)	2 (5.6)	
Poor QOL	13 (8.6)	1 (1.5)	6 (12.0)	6 (16.7)	
Disease stage (%) [b]					.238
Early	10 (6.6)	2 (3.1)	3 (6.0)	5 (13.9)	
Moderate	35 (23.2)	13 (20.0)	13 (26.0)	9 (25.0)	
Advanced	106 (70.2)	50 (76.9)	34 (68.0)	22 (61.1)	
MELD score [a]	16.8 (13.2-20.7)	17.4 (14.7-19.5)	14.3 (12.3-18.3)	19.9 (13.0-25.8)	.006
Creatinine (mg/dL) [a]	0.7 (0.6-0.9)	0.7 (0.6-1.0)	0.7 (0.6-0.8)	0.7 (0.6-0.9)	.528
Bilirubin (mg/dL) [a]	7.4 (3.6-15.7)	8.8 (4.1-15.1)	5.3 (2.6-10.6)	11.4 (3.9-20.7)	.091
INR [a]	1.2 (1.0-1.4)	1.1 (1.0-1.4)	1.0 (1.0-1.3)	1.3 (1.1-1.8)	.002
Albumin (g/L) [c]	30.9 (6.4)	29.7 (5.5)	30.8 (7.5)	33.0 (5.9)	.046

a.Shown as median (interquartile range)
b.Biochemical disease stage as defined by the Rotterdam criteria12
c. Shown as mean (standard deviation)
Missing values: Creatinine, n=0 (0%); Bilirubin, n=32 (21.1%); INR, n=9 (6%); Albumin, n=0 (0%); MELD score n=9 (6%).
Abbreviations: PBC, primary biliary cholangitis; AIH, auto immune hepatitis; HCC, hepatocellular carcinoma; QOL, quality of life; MELD, model for end-stage liver disease; INR, internationalized normal ratio.

Harms MH, van Buuren HR, Hansen BE, Metselaar HJ.
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CHAPTER 9
LIVER TRANSPLANTATION FOR PRIMARY BILARY CHOLANGITIS - THE NEED FOR TIMELY AND MORE EFFECTIVE TREATMENTS - AUTHORS' REPLY

Alimentary Pharmacology & Therapeutics, 2019

We would like to thank *Gerussi et al*[1] for their interest in our study and for highlighting valid remarks and questions that our paperraises[2]. Rightfully, the authors state that "non-response" to first-line therapy leaves patients prone to the need for liver transplantation (LT).Although- as previously proposed[3] - we believe the term "non-response" should be avoided and should rather be replaced with "incomplete response" given that these patients do in fact still benefit from ursodeoxycholic acid (UDCA) treatment, we would like to stress that our in-depth analyses in the Dutch population show that a minority of patients with primary biliary cholangitis (PBC) that are initially classified as "complete responders" after the first year of therapy, eventually also undergoes LT. This might be explained by the fact that some patients should actually be reclassified as "incomplete responders" when re-evaluating the response status during follow-up, as recently shown by *Goet et al*[4]. Secondly, the authors suggest that, in addition to epidemiological changes and a reduced rate of biochemical response in males, diagnostic delay might impact the increasing proportion of males transplanted for PBC. However, it seems unlikely that diagnostic delay in males has worsened over the past decades, leaving this as an implausible explanation for our finding. Another comment was that potential underreporting of autoimmune variant syndrome in PBC might impact our findings. Although research on the incidence and prevalence of such variant syndromes is limited, and studies on the impact of concomitant autoimmune hepatitis on long-term outcome are conflicting[5,6],exploring this further could indeed improve therapeutic strategies for this subgroup of patients with PBC, with a potential reduction in LTs as a result. Overall, we agree with *Gerussi et al* that our study stresses the unmet need of effective second-line therapies in PBC that reduce progression to liver failure, and the correct implementation of such drugs in clinical practice. As new potentially effective therapies including obeticholic acid and fibrates currently brighten the therapeutic horizon, we propose to renew this study after a decade or so, hopefully to find a renewed reduction in the absolute number of LTs for PBC.

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CHAPTER 10
GENERAL DISCUSSION & CONCLUSIONS

BACKGROUND

Primary biliary cholangitis (PBC) is a chronic disease that can lead to hepatic fibrosis which can eventually result in the development of cirrhosis. When left untreated, survival of patients with PBC is significantly impaired compared with the general population.

Because PBC is a rare disease with a relatively slow disease progression, research in the field of PBC has long been considered challenging. Over the past decade, large national and international collaborations have enabled the performance of high-quality research on big data with long-term follow-up in PBC. A previous landmark study performed by the *Global PBC Study Group* identified alkaline phosphatase (ALP) and bilirubin as independent surrogate markers of long-term clinical outcome[1]. This consensus has provided the field with an opportunity to assess the effect of potential therapeutic agents in a timely and ethical manner. Other important work is represented by the development of continuous risk scores[2, 3] based on readily available clinical parameters, correlating with a projected liver transplantation (LT)-free survival.

PROGNOSIS

Serum bilirubin has been extensively studied in terms of its association with long-term outcome in PBC[4]. Today it is a well-established marker of prognosis, both in UDCA-treated and untreated populations[5, 6]. *Shapiro et al.* already showed in 1979 that the behavioral pattern of bilirubin has two distinct phases: a first phase in which bilirubin is fairly stable for many years, followed by a phase with a rapid increase. Thus, since serum bilirubin is often not abnormal in the early stages of disease, it is often considered a 'late biomarker'. *Lammers et al.* showed that a threshold of 1.0 times the upper limit of normal (ULN) had the highest predictive ability[1]. This finding may have impacted the development of both inclusion criteria as well as primary endpoints of recent large clinical trials for second-line therapies, that included this ULN threshold for bilirubin[7, 8]. **Chapter 2** describes the prognostic relevance of serum bilirubin within the current normal range. We show that a threshold of 0.6 xULN has a good discriminative ability and that above 0.6 x ULN there is a linear relationship with risk of LT/death, both in patients that received UDCA treatment, as well as in patients that remained untreated. For patients with an initial serum bilirubin ≥0.6 x ULN, a reduction resulting in bilirubin <0.6 x ULN after one year of UDCA was associated with prolonged transplant-free survival. Our results imply that therapeutic goals may strive beyond 'normalization' and that future interventional studies could include patients with a bilirubin above 0.6 x ULN, and should consider this threshold as part of the primary or secondary endpoint. Although our study has mainly focused on serum bilirubin, we also show that ALP levels below 1.67 – a threshold previously used in biochemical response

criteria, and as inclusion criteria and endpoint in clinical trials[9, 10] – are associated with LT-free survival as well. Future studies are required to assess whether prognostic significance within the normal range can also be demonstrated for other biochemical measurements.

CIRRHOTIC COMPLICATIONS

Although today most patients are diagnosed in an early stage of disease, a fairly recent British study showed that up to 40% of patients had developed cirrhosis after 10 years of follow-up[11]. Subsequently, those patients are at risk of clinical complications that are associated with the presence of cirrhosis. Evidently, such decompensating events are associated with an increased risk of death or liver transplantation[12, 13]. Studies on these events in patients with PBC were rare, especially in patients treated with UDCA, as are the majority of PBC patients today. In **Chapter 3**, we studied non-neoplastic major cirrhosis-related complications including variceal bleeding, encephalopathy, and ascites in a UDCA-treated population. We found that overall, the 10-year cumulative incidence of first events was 9% and that in keeping with other liver diseases, subsequent survival was poor[14]. Reassuringly, however, this incidence decreased over time, which might be explained by increased awareness for PBC leading to diagnosis in early disease stages and by more prompt initiation of UDCA therapy after diagnosis. Stratification by both biochemical response and AST to Platelet Ratio Index (APRI) enabled us to identify a ~20% subgroup of patients with a very low long-term risk of complications. Our findings may be especially relevant for patient counseling, since PBC represents a condition in which patients are told to face serious and daunting disease-related risks. Risk factors for these complications were found to be largely the same as those related to liver transplantation (LT)-free survival[15-17], in which biochemical response is strongly associated with a reduced risk of LT and death. This further stresses the clinical importance of evaluating biochemical response to therapy.

THERAPIES AFFECTING THE NATURAL HISTORY

Ursodeoxycholic acid

The clinical picture of PBC was first described by Addison and Gull in 1851, followed by a long period of lack of therapeutic options[18]. After many disappointing clinical trials, UDCA was the first therapeutic agents showing promising results in absence of debilitating adverse events in the late 1980s. Several long-term cohort studies suggested that UDCA treatment was related to an improved LT-free survival, but based their conclusions on the comparison of observed survival versus predicted LT- survival according to prognostic models[19-21]. In contrast, randomized controlled trials repeatedly failed to show evidence of UDCA-induced survival benefit[22-31]. This led to a long-lasting discussion in the field of PBC experts, in which

doubts were recently substantiated by an in 2017 published Cochrane meta-analysis that concluded no demonstrated benefit of UDCA of LT and/or mortality[32]. In **Chapter 4**, the association between UDCA and LT-free survival was assessed by applying inverse probability of treatment weighting, a form of propensity matching aiming to emulate a clinical trial, to a cohort of both UDCA-treated and untreated patients with long-term follow-up. This resulted in a 10-year cumulative survival of 79.7% in UDCA-treated patients versus 60.7% in patients that remained untreated, corresponding to an overall hazard ratio of 0.46. The association remained significant in all subgroup analyses. Although the retrospective nature of this study results in the fact that a true causal relationship cannot be concluded, it is very difficult to think of any residual confounder that would completely diminish the cogent association that is found throughout all subgroup analyses. Importantly, we showed that the association between UDCA and prolonged survival remained in patients that were classified as inadequate responder. This implies that regardless of treatment response, UDCA should be continued. All previous response criteria stratified patients into '*responders'* versus '*non-responder'* or '*inadequate responders'*[15, 21, 33-37]. Giving the finding of therapeutic benefit in all patients, including those without full biochemical response, we propose to replace the aforementioned terms by '*incomplete response'* as this term is able to capture the remaining favorable therapeutic effect that these patients benefit from.

Therapeutic benefit can also be demonstrated as an absolute clinical efficacy measure, such as the number needed to treat (NNT) to prevent mortality (or LT). Assessment of the NNT is still uncommon in the field of hepatology and gastroenterology, but has recently gained popularity in other fields of medicine[38-40]. The NNT is easy to interpret for both patients and physicians, as it combines the relative risk reduction with the patients' baseline risk in one parameter. This is clinically meaningful, since both risks can differ between patients. In **Chapter 5**, we evaluated the clinical efficacy of UDCA in PBC. We found that that the overall NNT to prevent LT/death within 5 years was 11, which can be considered very low. In this respect it is important to consider that PBC is a chronic disease in which lifelong therapy is recommended. We therefore showed that the NNT further decreases over time, with an overall 10-year NNT of 6. We show that the variation in NNT with UDCA to prevent LT/death is mostly related to patient's a priori risk, as the relative risk reduction is fairly stable throughout subgroups. We were able to show how to estimate the NNT_{5y} for an individual patient based on their GLOBE score (a prognostic model previously developed by the Global PBC Study Group in UDCA-treated patients), as we show the GLOBE score also performs well in untreated patients. Together, our results in chapter 4 and 5 contribute to the body of evidence that justifies lifelong treatment with UDCA in all patients with PBC, especially in light of its favorable safety profile and relatively low costs. The current relevance of our results is stressed by reports of as much of 30% of Western cohorts of PBC patients remaining untreated yet today[41]. Moreover, our conclusions may aid physicians caring for patients with PBC in patient counseling and can support patient compliance.

Obeticholic acid

For decades, UDCA was the only approved therapeutic agent for PBC. Following the publication of the POISE trial[8], a phase III placebo-controlled trial of treatment with obeticholic acid (OCA), in 2016 this drug was approved by the FDA as the first second-line therapy for PBC. This was a milestone in the PBC landscape, especially since the approval process has been long and challenging, given the fact that the primary endpoint of the POISE trial was not based on hard clinical endpoints. The primary endpoint was based on ALP and bilirubin, which were considered to be surrogate markers for clinical outcome. The same year, both the Global PBC Study Group and the UK-PBC Study Group presented validated risk scores that correlated well with survival[2, 3]. In **Chapter 6**, we assessed the projected clinical benefit after one year of OCA, based on these risk scores that were applied to data from the POISE trial, in which OCA was used as add-on to UDCA in 93% of patients. Using the GLOBE score, OCA resulted in an estimated absolute risk reduction of LT/death of approximately 5% after 10 years, corresponding with a relative risk reduction of 24%. Similar results were obtained using the UK-PBC risk score. Although the primary endpoint was met by 47% of OCA-treated patients in the trial, we show that nearly all patients receiving OCA treatment show biochemical improvements that correspond with a significantly reduced estimated risk of adverse clinical outcome. This is also true for the patients who did not meet the trial primary endpoint for response. This finding underlines the importance of awareness of the limitations of using dichotomous response criteria or endpoints, especially when such results undergo subsequent cost-utility analyses[42] that could potentially have significant consequences for drug availability and/or reimbursement. Although the predictions in our study await confirmation by the ongoing phase 4 trial of OCA evaluating clinical outcomes in patients with PBC (COBALT; NCT02308111), a recent publication on the open-label POISE extension study shows promising results in which biochemical improvements were sustained up to 4 years[43].

Fibrates

After many years of pilot studies with either bezafibrates or fenofibrates as add-on treatment with promising results, a well-executed French placebo-controlled trial showed a spectacular improvement of biochemistry induced by add-on bezafibrate treatment associated with improved projected survival[7, 44]. Although some observational data is available, the results of an ongoing Chinese trial studying the effects of fenofibrates (NCT02965911) are awaited. No previous studies have compared the effects of these fibrate subtypes. In **Chapter 7**, we studied the effects of fibrate treatment in an international cohort of both fenofibrate- and bezafibrate-treated patients and assess potential differences. For both fibrate types, we found a beneficial effect on biochemistry, in keeping with previous studies. We did not identify large differences in the effects on either biochemistry or short-term clinical outcome. Although absolute numbers are small, a trend towards more side effects leading to treatment discontinuation in the fenofibrate group was a finding of interest that should

be validated in future studies. As the beneficial treatment effects do currently not indicate a preference for either fibrate subtype, side effects may represent a leading argument in the future. Today, however, the choice of fibrate type is often largely dictated by availability, as in many countries only one type is approved. Currently, different fibrate subtypes including PPAR-δ agonists with possible anti-fibrotic potential are being studied[45].

Treatment strategy

Discussing both OCA and fibrates as second-line add-on therapies raises the question which of these agents should qualify as first-choice agent. While well-executed placebo-controlled trials should provide more definitive answers, we are now lacking such information. Although symptom management is not within the scope of this thesis, the burden of disease-related symptoms is significant for patients with PBC[46]. Pruritus contributes to an impaired quality of life in many patients and effective treatment options are limited. While OCA is known to provoke or worsen pruritus in a proportion of patients[8], observational studies suggest a dramatic improvement in pruritus in patients treated with fibrates[47]. Although these promising findings await confirmation by a placebo-controlled trial[48] (FITCH; NCT02701166), effects on symptoms can be an important factor to take into account when considering second-line therapy. Interestingly, a pilot study showed that addition bezafibrate therapy was able to further improve both biochemistry and pruritus in patients treated with UDCA and OCA[49], suggesting that triple therapy should be further explored. However, it should be noted that at present, OCA is still the only officially approved second-line agent for PBC. Therefore, currently both physicians and patients have to be willing to prescribe/undergo off-label therapy with the associated risks when considering fibrates.

LIVER TRANSPLANTATION

Roughly up until 1980, PBC was the leading indication for LT in Europe, accounting for 30-50% of all LTs. Despite increasing disease prevalence[50, 51], today PBC is no longer a leading indication for LT. As the therapeutic horizon has markedly improved since then, prognosis is now much more favorable[7, 8, 19, 52-55]. However, in case of liver failure, liver transplantation remains the only therapeutic option to prevent premature death. As changes in selection criteria and the introduction of UDCA as an effective treatment likely impacted the relative importance of PBC as indication for LT, in **Chapter 8** we evaluated time trends in the number of liver transplantations for PBC across Europe over the past three decades. Although in line with previous smaller, older studies[56, 57] we found an initial gradual decrease in absolute and relative number of LTs for PBC, over the most recent decade we reported a stabilization in the absolute number of LTs for PBC where over 200 patients in Europe receive LT for PBC annually. The introduction of urgency-based allocation by MELD-score[58] has removed time on the waitlist as a contributing factor in liver allocation. This may have decreased

PBC patients' chances of receiving a LT giving the slowly progressive nature of this disease. Although outcome after LT for PBC is generally good, the remaining numbers of LT in PBC could be considered worrisome, especially since wait-list mortality was recently reported to be relatively high for patients with PBC as compared to other liver disease[59], suggesting that our number might be an underestimation of the actual need for LT.

CONCLUSIONS

Treatment with ursodeoxycholic acid is associated with a reduced risk of liver transplantation or death among all patients suffering from PBC. In absolute terms, the number needed to treat to prevent liver transplantation can be considered very low. Additionally, the large majority of UDCA-treated patients with PBC nowadays remain free of decompensating events. These findings justify the recommendation for lifelong treatment with UDCA for all patients with PBC. Nonetheless, an important group of PBC patients remains to have a diminished prognosis when compared to the normal population, and a subgroup still relies on LT to prevent premature death. Therefore, add-on treatment should be considered in case of an incomplete response to UDCA treatment. Second-line therapies include obeticholic acid, which induces improvement of biochemistry and prognosis as predicted by validated models, and fibrates, that also induce significant improvements in biochemistry. Because bilirubin within the currently accepted normal range is associated with LT-free survival, physicians may consider striving beyond normalization of bilirubin when evaluating treatment response.

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CHAPTER 11
NEDERLANDSE SAMENVATTING

Primaire biliaire cholangitis (PBC) is een chronische leverziekte die kan leiden tot fibrose en uiteindelijk cirrose. Wanneer patiënten niet behandeld worden is hun levensverwachting significant slechter dan de normale populatie.

Omdat PBC een zeldzame ziekte is die zich relatief langzaam ontwikkelt, is het verrichten van onderzoek naar deze ziekte zeer lastig gebleken. Gedurende het afgelopen decennium hebben grote nationale en internationale samenwerkingsverbanden – zoals de *Global PBC Study Group* - het mogelijk gemaakt om accuraat onderzoek te doen naar lange termijn uitkomsten in grote groepen patiënten met PBC. Een eerdere studie verricht door de *Global PBC Study Group* toonde aan dat alkalisch fosfatase en bilirubine onafhankelijke surrogaatmarkers zijn voor klinische uitkomstmaten zoals levertransplantatie en overlijden. Deze consensus leidde tot de mogelijkheid om de effecten van potentieel therapeutische middelen te onderzoeken binnen een relatief kort tijdsbestek. Ook de ontwikkeling van continue risico scores die zijn gebaseerd op beschikbare klinische parameters, waren een belangrijke stap voorwaarts.

PROGNOSE

Bilirubine is uitgebreid onderzocht als voorspeller voor klinische uitkomstmaten. Tegenwoordig is het een geaccepteerde marker voor prognose, zowel in onbehandelde patiënten als in patiënten behandeld met ursodeoxycholzuur (UDCA). *Shapiro et al.* beschreven reeds in 1979 dat het patroon van bilirubine wordt gekenmerkt door twee duidelijk afgrensbare fases: een eerste fase waarin het bilirubine zich redelijk stabiel gedraagt gedurende een ruim aantal jaren, gevolgd door een fase met een snelle stijging. Omdat bilirubine gedurende de vroege ziektestadia meestal niet abnormaal is, wordt het meestal beschouwd als een ‘late biomarker’. *Lammers et al.* hebben eerder beschreven dat een grens van 1.0 x de bovengrens van normaal de beste voorspellende waarde voor levertransplantatie of overlijden had. Deze bevinding heeft mogelijk impact gehad op de ontwikkeling van zowel inclusiecriteria als primaire uitkomstmaten van klinische studies voor tweedelijns therapeutische middelen, waarin deze grens voor bilirubine frequent is opgenomen. In **hoofdstuk 2** wordt de prognostische relevantie van bilirubine binnen de huidige grenzen van normaal beschreven. We laten zien dat een grens van 0.6 x de huidige bovengrens van normaal goed in staat is risico te discrimineren, met een lineaire relatie. Dit geldt zowel voor onbehandelde patiënten als voor patiënten behandeld met UDCA. We demonstreren tevens dat voor patiënten die initieel een bilirubine boven de 0.6 x de huidige bovengrens hebben, een therapie-geïnduceerde verlaging tot onder die grens is geassocieerd met een verbeterde transplantatie-vrije overleving. Deze resultaten suggereren dat we met therapie mogelijk moeten streven naar een niveau verder dan alleen ‘normalisatie’ en dat voor toekomstige interventiestudies overwogen kan worden om

patiënten met een bilirubine boven 0.6x de huidige bovengrens te includeren. Ook zou deze grens overwogen kunnen worden als onderdeel van primaire of secundaire uitkomstmaten. Alhoewel deze studie zich primair heeft gefocust op bilirubine, laten wij tevens zien dat alkalisch fosfatase onder de 1.67 x de huidige bovengrens – een grens die frequent is gebruikt in biochemische responscriteria, evenals als inclusiecriterium en eindpunt – ook geassocieerd is met transplantatie-vrije overleving. Toekomstige studies moeten uitwijzen of andere biochemische markers ook prognostisch zijn binnen de huidige range van normaal.

COMPLICATIES VAN CIRROSE

Alhoewel tegenwoordig de meeste PBC-patiënten worden gediagnosticeerd in een vroeg ziektestadium, liet een recente Britse studie zien dat circa 40% van alle patiënten cirrose ontwikkelt na tien jaar follow-up. Deze patiënten hebben vervolgens een verhoogd risico op het ontwikkelen van klinische complicaties die zijn geassocieerd met het hebben van cirrose, die op hun beurt een toegenomen risico op levertransplantatie en overlijden met zich mee brengen. In **hoofdstuk 3** hebben we onderzoek gedaan naar niet-neoplastische, cirrose-gerelateerde klinische complicaties. Hieronder vielen het ontwikkelen van ascites, een varicesbloeding en hepatische encefalopathie. We beschrijven dat de cumulatieve 10-jaars incidentie van eerste complicaties 9% was in de gehele studiepopulatie en dat, net als bij andere leverziekten, de prognose na dergelijke complicaties slecht was. Desalniettemin vonden we dat de incidentie van deze complicaties de afgelopen jaren is afgenomen, wat mogelijk verklaard kan worden door verbeterde herkenning van het ziektebeeld waarbij therapie sneller gestart wordt. We vonden dat circa 20% patiënten zowel een goede biochemische respons op ursodeoxycholzuur als een lage APRI-score (AST to Platelet Ratio Index) hadden. Deze groep patiënten had een zeer lage kans op het ontwikkelen van de eerdergenoemde klinische complicaties. Deze bevindingen kunnen relevant zijn voor het voorlichten van patiënten in de spreekkamer. De risicofactoren die in deze studie geassocieerd waren met het optreden van cirrose-gerelateerde complicaties zijn grotendeels vergelijkbaar met eerder beschreven risicofactoren voor levertransplantatie en overlijden, waarbij we vonden dat biochemische respons zeer sterk geassocieerd was. Die bevinding benadrukt het belang van het evalueren van biochemische respons op therapie.

BEHANDELINGEN DIE DE PROGNOSE VERANDEREN

Ursodeoxycholzuur

Het klinisch beeld van PBC werd voor het eerst beschreven door *Addison en Gull* in 1851, waarna een lange tijd volgde waarin er geen therapeutische opties waren voor de ziekte. Na vele teleurstellende studies die de werkzaamheid van verschillende medicijnen evalueerden,

was ursodeoxycholzuur eind jaren ’80 het eerste medicijn dat veelbelovende resultaten opleverde zonder dat er hevige bijwerkingen optraden. Verschillende lange termijnstudies wekten de indruk dat ursodeoxycholzuur leidde tot een verbeterde levensverwachting, maar deze aannames waren slechts gebaseerd op de vergelijking van geobserveerde levensduur met geschatte levensduur op basis van prognostische modellen. Daar tegenover stond dat diverse gerandomiseerde studies geen gunstig effect op de levensverwachting konden aantonen. Dit leidde tot een langdurige discussie tussen experts op het gebied van PBC. De twijfels aan de werkzaamheid van ursodeoxycholzuur werden ondersteund door een Cochrane meta-analyse die in 2017 verscheen, waarin werd geconcludeerd dat er geen bewijs was voor winst in levensverwachting door behandeling met ursodeoxycholzuur. In **hoofdstuk 4** onderzochten we de associatie tussen ursodeoxycholzuur therapie en transplantatie-vrije overleving door het toepassen van een moderne statistische methode: inverse probability of treatment weighting. Dit is een vorm van propensity matching die als doel heeft in retrospect een gerandomiseerde studie na te bootsen. Dit was mogelijk door de beschikbaarheid van een groot cohort van zowel behandelde als onbehandelde patiënten met lange termijn follow-up data. Dit resulteerde in een cumulatieve transplantatie-vrije overleving van 79.7% in behandelde patiënten, versus 60.7% in onbehandelde patiënten, corresponderend met een hazard ratio van 0.46. Deze associatie was significant in alle subgroepen van patiënten. Alhoewel bij een retrospectieve studie ware effectiviteit nooit bewezen kan worden, is het bijzonder moeilijk om een residual confounder te bedenken die deze associatie geheel teniet zou doen. Van belang is dat we tevens demonstreerden dat de overlevingswinst ook werd aangetoond in patiënten die volgens internationale criteria werden geclassificeerd als ‘onvoldoende of niet responderend op behandeling’. Met oog op de bevinding dat overlevingswinst werd gezien bij alle behandelde patiëntgroepen, stellen wij voor de voorgenoemde termen ‘onvoldoende of niet responderend’ te vervangen door ‘incompleet responderend’. De afwezigheid van het vinden van therapie-gerelateerde overlevingswinst in de vroegere gerandomiseerd studies wordt waarschijnlijk verklaard door een insufficiëntie follow-up bij deze langzaam progressieve ziekte.

Therapeutisch voordeel kan ook worden geëvalueerd gemeten in een absolute klinische effectmaat, zoals de *number needed to treat (NNT)* om overlijden of levertransplantatie te voorkomen. Binnen de hepatologie is het gebruik van deze effectmaat nog relatief zeldzaam, maar in andere medische velden is dit al jaren gebruikelijk. De NNT is gemakkelijk te interpreteren voor zowel patiënten als zorgverleners omdat het de relatieve risicoreductie en het risico van de patiënt wanneer er geen behandeling plaatsvindt, vangt in één parameter. Dit is klinisch van groot belang, omdat die beide factoren kunnen verschillen van patiënt tot patiënt. **Hoofdstuk 5** beschrijft de klinische effectiviteit van ursodeoxycholzuur in patiënten met PBC. We vonden dat in de gehele studiepopulatie, de NNT om één overlijden of levertransplantatie te voorkomen binnen vijf jaar therapie 11 was. Dit wordt over het algemeen als zeer laag beschouwd. Het is belangrijk om te beseffen dat PBC een chronische

ziekte is waar levenslange therapie wordt aangeraden, waarbij de grens van vijf jaar dus meestal ruimschoots overschreden wordt. We zagen dat de NNT met de tijd afnam, waarbij de NNT_{10jaar} slechts 6 bleek. We demonstreerden bovendien dat de variatie in NNT vooral afhankelijk was van het risico op een klinisch eindpunt van de onbehandelde patiënt, terwijl de relatieve risicoreductie geïnduceerd door ursodeoxycholzuur vrij stabiel was. Daarnaast laten we zien hoe de NNT voor de individuele patiënt geschat kan worden op basis van de GLOBE score (een prognostisch model eerder ontwikkeld door de Global PBC Study Group in behandelde patiënten), door onder andere aan te tonen dat de GLOBE score ook betrouwbaar de levensverwachting voorspelt in onbehandelde patiënten. De resultaten uit hoofdstuk 4 en 5 dragen gezamenlijk bij aan het bewijs dat het advies om patiënten met PBC levenslang te behandelen met ursodeoxycholzuur ondersteunt, zeker gezien de afwezigheid van belangrijke bijwerkingen en de lage kosten. Het hedendaagse belang van deze resultaten werd benadrukt door een recente studie die toonde dat in een Westerse populatie circa 30% van de patiënten met PBC onbehandeld bleven. Ook kunnen onze studieresultaten artsen ondersteunen in de voorlichting van patiënten in de spreekkamer en kan het de therapietrouw mogelijk verbeteren.

Obeticholzuur

Jarenlang was ursodeoxycholzuur het enige geregistreerde medicijn voor PBC. Na de publicatie van de POISE studie, een fase III placebo-gecontroleerde studie waarin obeticholzuur werd geëvalueerd, werd dit middel in 2016 goedgekeurd door de FDA. Dit was een mijlpaal in het PBC landschap, zeker gezien de goedkeuring gebaseerd was op een surrogaat eindpunt van alkalisch fosfatase en bilirubine in plaats van op harde eindpunten. Ditzelfde jaar publiceerden zowel de Global PBC Study Group als de UK-PBC Study Group gevalideerde risicoscores die goed correleerden met levensverwachting. In **hoofdstuk 6** evalueerden we de geschatte overlevingswinst geïnduceerd door obeticholzuur, door de uitkomsten van de POISE studie toe te passen op deze risico scores. Op basis van de GLOBE score resulteerde obeticholzuur in een absolute risicoreductie van circa 5% na 10 jaar op levertransplantatie of overlijden, corresponderend met een relatieve risicoreductie van 24%. Vergelijkbare resultaten werden gevonden met gebruik van de UK-PBC Risk score. Alhoewel het primaire eindpunt van de studie door slechts 47% van de behandelde patiënten werd gehaald, vonden wij dat nagenoeg alle patiënten die waren behandeld met obeticholzuur verbeteringen van biochemie toonden, waarmee ze bovendien een significante geschatte overlevingswinst behaalden. Deze bevinding onderstreept het belang te beseffen wat de beperkingen zijn van het gebruik van dichotome uitkomstmaten, zeker wanneer er op basis van dergelijke resultaten ook kosten-effectiviteitstudies worden verricht die potentieel verstrekkende gevolgen kunnen hebben op de beschikbaarheid en vergoeding van middelen. Alhoewel onze resultaten nog in afwachting zijn van klinische bevestiging door de momenteel lopende fase vier studie, liet een recente publicatie van de open-label POISE-extensiestudie zien dat de biochemische respons blijvend was gedurende 4 jaar.

Fibraten

Na vele jaren waarin verscheidene pilotstudies met bezafibraten of fenofibraten als add-on behandeling naast ursodeoxycholzuur veelbelovende resultaten lieten zien, demonstreerde een goed uitgevoerde Franse placebogecontroleerde studie spectaculaire biochemische verbeteringen door behandeling met bezafibraten met een verbeterde geschatte overleving tot gevolg. Er zijn tot op heden geen grote placebogecontroleerde studies die fenofibraten evalueren gepubliceerd, noch zijn er studies die de effecten van deze twee subtypes hebben vergeleken. In **hoofdstuk 7** onderzochten we de effecten van behandeling met fibraten in een internationaal cohort van patiënten die ofwel met bezafibraten, ofwel met fenofibraten waren behandeld en evalueerden we eventuele verschillen. Bij beide subtypes vonden we een gunstig effect op de biochemie, in lijn met eerdere studies. We vonden geen grote verschillen in het effect op biochemie en korte termijn klinische uitkomsten. Alhoewel de aantallen beperkt waren, zagen we een trend richting meer discontinuatie van therapie leidende bijwerkingen in de groep die werd behandeld met fenofibraten. Deze bevinding behoeft verdere validatie in toekomstige studies en is van belang omdat bij een gebrek aan belangrijke verschillen in effect, het bijwerkingenprofiel leidend zou kunnen zijn in de keuze voor het type fibraat. Momenteel wordt de keuze echter vaak bepaald door beschikbaarheid, gezien in veel landen slechts één type fibraat verkrijgbaar is. Momenteel worden ook andere fibraat subtypes in studies geëvalueerd, waaronder PPAR-δ agonisten met mogelijke anti-fibrotische effecten.

Therapeutische strategie

Het bespreken van zowel obeticholzuur als fibraten als tweedelijns add-on behandeling leidt tot de vraag welke van deze middelen de eerste keus verdient. Goed uitgevoerde placebogecontroleerde studies zouden definitieve antwoorden kunnen verstrekken, maar die zijn tot op heden niet voor handen. Alhoewel symptoommanagement niet het focus is van dit proefschrift, is de ziektelast van PBC-gerelateerde symptomen aanzienlijk. Jeuk draagt bij aan een verminderde kwaliteit van leven voor veel patiënten. In veel gevallen zijn de beschikbare therapeutische opties hiervoor beperkt. Van obeticholzuur is bekend dat het jeuk kan induceren of verergeren, terwijl fibraten lijken te leiden tot een drastische verbetering van de jeukklachten. Alhoewel we de resultaten van een Nederlandse gerandomiseerde studie naar het effect van bezafibraten op jeuk nog afwachten, kan het effect op symptomen een belangrijke factor zijn in de keuze voor een tweedelijns medicament. Preliminaire resultaten van een Belgische studie toonden dat het toevoegen van bezafibraten bij patiënten die reeds behandeld worden met ursodeoxycholzuur én obeticholzuur, leidde tot zowel een biochemische verbetering als een afname van de jeuk. Dit wekt de suggestie dat eventuele triple therapie verder geëxploreerd moet worden. Desalniettemin moet genoemd worden dat tot op heden obeticholzuur het enige geregistreerde tweedelijns medicijn is voor PBC. Zowel patiënten als artsen moeten momenteel derhalve bereid zijn om *off-label* medicijnen in te nemen of voor te schrijven, wanneer fibraten overwogen worden.

LEVERTRANSPLANTATIE

Tot rond 1980 was PBC de meest voorkomende indicatie voor levertransplantatie in Europa, verantwoordelijk voor 30-50% van alle transplantaties. Ondanks een toenemende ziekteprevalentie is PBC tegenwoordig geen veel voorkomende indicatie meer. Omdat de herkenning van de ziekte verbeterd is en de therapeutische horizon voor PBC sinds die tijd uitgebreid is, is de prognose van patiënten met PBC sterk verbeterd. Desalniettemin is in het geval van leverfalen, levertransplantatie nog altijd de enige optie om overlijden te voorkomen. Omdat zowel veranderingen in selectiecriteria als de introductie van ursodeoxycholzuur waarschijnlijk een belangrijke impact hebben gehad op het relatieve belang van PBC als indicatie voor transplantatie, onderzochten wij in **hoofdstuk 8** de veranderingen over de tijd in het absolute en relatieve aantal levertransplantaties voor PBC in Europa gedurende de afgelopen 30 jaar. In lijn met eerdere studies vonden wij gedurende de eerste 20 jaar een geleidelijke afname in zowel absolute als relatieve aantallen levertransplantaties bij patiënten met PBC. De laatste 10 jaar bleek er echter sprake van een stabilisatie in het absolute aantal levertransplantaties voor PBC, waarbij jaarlijks ca. 200 PBC-patiënten levertransplantatie ondergingen. Met de introductie van het urgentie-gebaseerde allocatiemodel middels de MELD score was tijd op de wachtlijst niet langer een bijdragende factor voor toewijzing van een lever. Dit zou ongunstig kunnen zijn voor patiënten met PBC met oog op de langzame progressie van deze ziekte. Alhoewel overleving na transplantatie bij patiënten met PBC over het algemeen goed is, is het zorgelijk dat het absolute jaarlijkse aantal levertransplantaties niet daalt, zeker omdat recente studies vermelden dat de wachtlijstmortaliteit relatief hoog is onder patiënten met PBC. Dit wekt de suggestie dat de behoefte aan levertransplantaties voor PBC mogelijk onderschat wordt.

CONCLUSIES

Behandeling met ursodeoxycholzuur is geassocieerd met een afgenomen risico op levertransplantatie en overlijden voor patiënten met PBC. De number needed to treat om een levertransplantatie of overlijden te voorkomen kan beschouwd worden als zeer laag. De grote meerderheid van patiënten die behandeld worden met ursodeoxycholzuur ontwikkelt tegenwoordig geen leverdecompensatie. Deze bevindingen rechtvaardigen het advies om patiënten met PBC levenslang te behandelen met ursodeoxycholzuur. Desalniettemin heeft een substantieel deel van de patiënten ondanks behandeling met ursodeoxycholzuur een prognose die slechter is dan de gewone populatie en is een subgroep van deze patiënten nog altijd afhankelijk van levertransplantatie om vervroegd overlijden te voorkomen. Daarom moet tweedelijns behandeling overwogen worden in geval van een incomplete respons op ursodeoxycholzuur. In dat geval kunnen zowel obeticholzuur als fibraten overwogen worden, die beide leiden tot verbetering van biochemie en geschatte overleving. Omdat

bilirubine binnen de huidige grenzen van normaal is geassocieerd met transplantatie-vrije overleving, kan worden overwogen om voorbij normalisatie te streven wanneer respons op therapie geëvalueerd wordt.

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Allereerst, mijn kamergenootjes! Shannon en Els, tijdens mijn eerste MDL skiweekend legden we de basis voor een fantastische tijd op het dak en een vriendschap voor lang daarna. *Shan*, mijn kamergenootje vanaf dag één. Het klikte meteen en er zijn weinig mensen waar ik zo intens hard mee kan lachen (om dingen die niemand anders begrijpt..). Wij hebben eindeloos veel gepraat maar hebben eigenlijk vaak aan een blik al genoeg. Gelukkig heb je onderweg ingezien dat Rotterdam toch echt veel leuker is en werden we zelfs herenigd in het YSL! Ik mis je enorm sinds je daar weg bent. Lieve *Elsie*, na een jaar pikte ik de plek van WP in op 425. In de twee jaar die volgden heb jij voorgoed een figuurlijk plekje in mijn hart veroverd, en gelukkig zelfs ook een letterlijk plekje om de hoek! Dankjewel dat jij geen genoegen neemt met een half antwoord en altijd doorvraagt naar dingen die belangrijk zijn, binnen het onderzoek maar vooral ook daar buiten. Te gek dat je vandaag naast mij wil staan. Lieve *Sophia*, toen jij op het dak verscheen wist ik meteen dat wij een match zouden zijn. We bleken neighbours in Kralingen en werden zelfgekozen roomies op het dak. Het was een voorrecht om mijn laatste tijd op het dak met jou te delen, jij hebt me er absoluut doorheen gesleept! Dat jij vandaag mijn paranimf bent is metaforisch voor hoe jij er altijd bent, in spannende of moeilijke maar ook in te gekke tijden, dankjewel.

Lieve *Joan*, jouw schaterlach, die regelmatig door de muur heen te horen was, maakt me altijd vrolijk. Ik mis je, oude Amsterdammer! *Louisa*, lieve Lies, partner in crime, team nooit meer naar huis is nooit compleet zonder jou. Dank voor de talloze geniale feestjes waarbij we de lichten uit (en weer aan..?) hebben gedaan en alle andere momenten die we hebben gedeeld. Ik kijk enorm uit naar onze hereniging in het EMC! Lieve *Raoel*, twee superleuke periodes in Toronto kwam ik jou vergezellen en ontdekte ik hoe leuk jij bent! Helaas hebben

we het YSL niet samen meegemaakt, maar gelukkig is een gezamenlijke voorliefde voor lekker eten en drinken een solide basis van onze vriendschap. Lieve *Sil*, jij bent altijd overal voor in en het lijkt wel alsof er 30 uren in jouw dag zitten, om jaloers van te worden! Bedankt voor jouw onuitputtelijke energie en goede vibe! Lieve *Stella,* jouw positieve instelling en uitstraling zijn uniek. Bedankt dat je me altijd vrolijk maakt! Lieve *Fanny*, jouw werkethos is ongekend. Gelukkig kun je net zo hard borrelen als werken, bedankt voor alle gezelligheid! Lieve *Juul*, hoe fijn dat jij team MDL YSL bent komen versterken. Ellendige einden hardlopen, fijne gesprekken met een goed glas wijn, slechte gifjes en mooie feestjes, met jou kan alles! *Wim*, dank dat ik mocht verder werken op het fundament wat jij samen met Henk en Bettina gelegd hebt en voor alle heerlijk droge grappen. *Jorn*, bedankt voor de gezellige samenwerking. Heel veel succes nog even, de finish is in zicht! Lieve *Rozanne,* wij bleken van hetzelfde hout gesneden. Zo trof ik in jou niet alleen een ideale opvolger, maar ook een nieuwe vriendschap. Zowel PBC-inhoudelijk als op de borrels gaan we de er de komende jaren iets moois van maken, ik heb er zin in! Alle andere lieve lab-ratten en dak-duiven: dank voor deze te gekke tijd, ik heb van jullie genoten! Gelukkig komen we elkaar ongetwijfeld snel weer tegen.

Lieve *YSL collega's*, dank voor het herstellen van mijn vertrouwen in de interne geneeskunde door mij te laten zien dat ook daar de congressen en skiweekenden onvergetelijk zijn! Daarnaast dank ik jullie natuurlijk dat jullie mij na die jaren van vooral wetenschap wegwijs hebben gemaakt in de kliniek en voor de ruimte die ik kreeg om mijn proefschrift af te ronden en krijg om mijn wetenschappelijke ambities voort te zetten. Vanaf nu heb ik meer tijd voor borrels!

En dan mijn familie en vrienden, de mensen die mij maken tot wie ik ben en wie ik wil zijn. Jullie wil ik heel graag bedanken. Zonder iemand tekort te willen doen, wil ik een aantal mensen in het speciaal noemen.

Lieve *Tanja*, lieve roomie. Vier hele mooie jaren hebben wij gedeeld in het mooiste paleisje van Blijdorp, waar we stuivertje wisselden met coschappen en promoveren. Ik heb hele bijzondere herinneringen aan die tijd. Dat we elkaar de komende jaren dankzij jouw ongekende doorzettingsvermogen ook op onderwijsmomenten en MDL-congressen zullen zien is de perfecte bonus op onze vriendschap! Lieve *Edmée*. Jouw trouwheid is ongeëvenaard, er is niemand anders van wie ik zo attent op alle momenten die voor mij belangrijk waren een kaartje, telefoontje, berichtje of andersoortig steuntje in de rug kreeg. Lieve ladies, gelukkig zijn onze vriendschappen zeker niet afhankelijk van deze Maasstad en weten we elkaar, ondanks wat extra kilometers, heel goed te vinden.

Lieve *Charlotte, Inge en Lisanne*. Wat ben ik blij dat ik jullie na al die jaren nog steeds mag scharen onder mijn dierbaarste vrienden. We hebben over de jaren bijzondere tijden met elkaar doorgebracht en dat bleek het fundament voor een vriendschap voor het leven. Nog altijd kom ik vol energie weer terug van waar we elkaar dan ook zien.

Ruben, lieve Ruubje. Jouw ambities en je hang naar altijd meer en beter (getuige je boekje/promotiebijbel) zijn aanstekelijk. Met jou kan ik discussiëren over wetenschap, eindeloos over niks praten en daarnaast alles bespreken waar het echt over gaat in het leven. Dank voor die fijne vriendschap.

Lieve *Yagmur, Roos en Bodine*. Dank jullie wel voor alle welkome promotie-afleidingsmomenten. Ondanks dat wij initieel verbonden zijn door het dokter-zijn, gaat het (gelukkig) vooral vaak over andere dingen. Rio was natuurlijk legendarisch, maar ik weet zeker dat er nog vele onvergetelijke momenten zullen volgen. Ik ben heel dankbaar dat ik vriendinnetjes als jullie heb!

En dan jij, lieve *papa*. Jij zal voor mij altijd een voorbeeld blijven en waarschijnlijk was jij het die mij ergens in mijn achterhoofd motiveerde om dit promotietraject aan te gaan en door te zetten. Hoe jij moeilijke vraagstukken wist om te zetten in dagelijkse, vaak supermarkt-gerelateerde metaforen en daarnaast een bijna grenzeloze passie had voor patiëntenzorg, was uniek. Ik mis je.

Lieve hele grote broer, lieve *Job*. Je daagt me vaak uit met ingewikkelde raadsels en vraagstukken en bent altijd benieuwd naar mijn visie. Je enthousiasme voor wetenschap is inspirerend! Dank daarvoor (en voor je eindeloze geduld als ik voor de honderdste keer mijn telefoon niet opneem). Nu maar hopen dat ik niet te veel onder doe voor jouw verdediging… Lieve *Anne-Marie*, dankjewel dat je Job zo gelukkig maakt. Hoe leuk dat ik Rio ook met jou kon beleven, in de toekomst komen daar vast nog allerlei avonturen bij!

Jorijn, liefste zus, jij bent als grote zus degene waar ik al zo lang ik me kan herinneren stiekem tegen op kijk omdat jij altijd je eigen, originele pad kiest en nooit bang lijkt voor avontuur. Jouw blik op de wereld is out of the box en daardoor heel verfrissend. Dankjewel dat jij er ongeacht fysieke afstand altijd voor mij was en bent. Zo fijn dat je nu weer in de buurt bent!

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**joint first authorship*

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Thomas Smith

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Maren Hermine Harms werd geboren op 28 maart 1990 te Bennebroek. Nadat zij in 2007 haar VWO-diploma behaalde aan het Hageveld College te Heemstede, startte zij met haar studie geneeskunde in Rotterdam. Tijdens de doctoraalfase liep Maren stage op de Intensive Care van het Red Cross War Memorial Children's Hospital in Kaapstad en werkte als student-assistent op de afdelingen psychiatrie, gynaecologie en op de huisartsenpost. Ze verrichtte haar afstudeeronderzoek naar patiëntkarakteristieken en motivatie van zelfverwijzers op de afdeling Spoedeisende Hulp Geneeskunde van het Erasmus MC. Na het doorlopen van haar coschappen behaalde Maren in 2014 cum laude haar artsexamen. Aansluitend startte ze met haar promotieonderzoek naar de prognose en behandeling van primaire biliaire cholangitis, onder leiding van prof. dr. H.J. Metselaar, dr. H.R. van Buuren en dr. B.E. Hansen. Tijdens haar promotieonderzoek was ze tevens actief binnen Promovendi Netwerk Nederland en was zij geruime tijd voorzitter van Promeras, het vertegenwoordigend orgaan van alle promovendi binnen het Erasmus MC. In 2018 is ze gestart met de opleiding tot Maag-, Darm- en Leverarts (opleider: prof.dr. C.J. van der Woude) en ontving ze de NVH Young Hepatologist Award van de Nederlandse Vereniging voor Hepatologie voor het beste klinisch hepatologisch wetenschappelijk artikel van Nederlandse bodem in 2018. Momenteel doorloopt zij haar tweejarige vooropleiding Interne Geneeskunde in het IJsselland Ziekenhuis in Capelle aan de IJssel (opleiders: dr. H.E. van der Wiel en dr. E.L.E. de Bruijne). Maren woont samen met Rutger in Rotterdam.

Maren Hermine Harms was born in Bennebroek (The Netherlands) on March 28, 1990. After graduating high school, she started studying Medicine in Rotterdam. She took an internship at the Intensive Care Unit of the Red Cross War Memorial Children's Hospital in Cape Town and worked as student assistant at the Psychiatry and Gynecology wards and at the out of hours General Practitioners Clinic. For her master thesis she researched patient characteristics of and reasons for self-referral at the Emergency Department of the Erasmus MC. After finishing her rotations in 2014, she graduated with honor. Subsequently, she started working on her doctorate thesis regarding the prognosis and treatment of primary biliary cholangitis, under the supervision of prof. dr. H.J. Metselaar, dr. H.R. van Buuren and dr. B.E. Hansen. During this time, she participated in PhD Network Netherlands and served as chairwoman of Promeras, the representing body of all PhD students in the Erasmus Medical Center. In 2018 she started her specialization to become a gastroenterologist and received the NVH Young Hepatologist Award for the best Dutch clinical hepatology paper in 2018. Currently she is working as an Internal Medicine resident at the IJsselland Hospital in Capelle aan de IJssel. Together with Rutger, Maren lives in Rotterdam.

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PhD student	Maren H. Harms
Department	Gastroenterology and Hepatology
Promotor	Prof.dr. H.J. Metselaar
Copromotors	Dr. H.R. van Buuren & Dr. B.E. Hansen

1. PhD training	Year	Workload
Methodology and Biostatistics Courses		
Journal clubs, department of gastroenterology/hepatology, Erasmus MC, Rotterdam	2014 - 2017	60 hours
Endnote workshop, Erasmus MC library, Rotterdam	2014	6 hours
PubMed workshop, Erasmus MC library, Rotterdam	2014	6 hours
Biostatistics for clinicians, Netherlands institute for Health Sciences (NIHES), Rotterdam	2014	40 hours
Presenting skills for junior researchers, Erasmus Postgraduate School for Molecular Medicine (MolMed), Erasmus MC, Rotterdam	2015	12 hours
Biomedical English writing and communication, Erasmus MC, Rotterdam	2015	40 hours
Course Joint Modeling, Erasmus Summer School, Rotterdam	2016	24 hours
Research Integrity Courses		
BROK cursus, consultatiecentrum patiëntgebonden onderzoek, Erasmus MC, Rotterdam	2014	24 hours
Integrity in scientific research, dept. of Medical Ethics and philosophy, Erasmus MC, Rotterdam	2015	36 hours
Oral presentations		
Incidence and Impact of Decompensating Events in PBC, Half-yearly Meeting of the Dutch Association of Hepatology (NVH), Veldhoven	2015	12 hours
Incidence and Impact of Decompensating Events in Primary Biliary Cholangitis, The Liver Meeting, 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, USA	2015	36 hours
Behavioral Patterns of Total Serum Bilirubin Prior to Major Clinical Endpoints in PBC, 50th International Liver Conference of the European Association for the Study of the Liver (EASL), Barcelona, Spain	2016	24 hours
Improvement in Estimated Survival after 1 Year of Obeticholic Acid Treatment, 50th International Liver Conference of the EASL, Barcelona, Spain	2016	24 hours
Primary Biliary Cholangitis at a Young Age - Clinical Characteristics and Prognosis, Half-yearly Meeting of the Dutch Association of Hepatology, Veldhoven	2016	12 hours
Comparable Beneficial Effects of Beza- and Fenofibrate in Primary Biliary Cholangitis, The Liver Meeting, 67th Annual Meeting of the AASLD, Boston, USA	2016	36 hours
Time Trends in Major Hepatic Complications in Primary Biliary Cholangitis: incidence and transplant-free survival, Digestive Disease Days, Veldhoven	2017	12 hours

1. PhD training	Year	Workload
Time Trends in Major Hepatic Complications in Primary Biliary Cholangitis: incidence and transplant-free survival, The Liver Meeting, 68th Annual Meeting of the AASLD, Washington, USA	2017	36 hours
Ursodeoxycholic acid is Associated with Prolonged Transplant-free Survival in Primary Biliary Cholangitis, 52th International Liver Conference of EASL, Paris, France	2018	36 hours

Poster presentations		
Risk Factors for Hepatic Decompensation in Primary Biliary Cirrhosis, 49th International Liver Conference of the EASL, Vienna, Austria	2015	12 hours
Risk Factors for Hepatic Decompensation in Primary Biliary Cirrhosis, Falk Symposium 197, Autoimmune Liver Diseases, Lisbon	2015	12 hours
Primary Biliary Cholangitis at a Young Age - Clinical Characteristics and Prognosis, The Liver Meeting, 67th Annual Meeting of the AASLD, Boston, USA	2016	12 hours
Association between UDCA Therapy and Prolonged Transplant-free Survival among Patients with Primary Biliary Cholangitis, AASLD, Boston, USA	2016	12 hours
Risk reduction with Obeticholic Acid in patients not achieving the POISE primary endpoint, 51th International Liver Conference of the EASL, Amsterdam	2017	12 hours
Effect of Obeticholic Acid Treatment in patients with PBC on categorical shifts in GLOBE score, 51th International Liver Conference of the EASL, Amsterdam	2017	12 hours
Risk reduction with Obeticholic Acid in patients not achieving the POISE primary endpoint, Monothematic conference, Cholangiocytes in health and disease, EASL, Oslo, Norway	2017	12 hours

Attended (inter)national conferences		
The Liver Meeting, 65th Annual Meeting of the American AASLD, Boston, USA	2014	28 hours
50th International Liver Conference of the EASL, Vienna, Austria	2015	28 hours
Falk Symposium 197, Autoimmune Liver Diseases, Lisbon, Portugal	2015	20 hours
Half-yearly Meeting of the Dutch Association of Hepatology, Veldhoven	2015	12 hours
The Liver Meeting, 66th Annual Meeting of the AASLD, San Francisco, USA	2015	28 hours
Half-yearly Meeting of the Dutch Association of Gastroenterology, Veldhoven	2016	12 hours
51st International Liver Conference of the EASL, Barcelona, Spain	2016	28 hours
Half-yearly Meeting of the Dutch Association of Hepatology, Veldhoven	2016	12 hours
The Liver Meeting, 67th Annual Meeting of the AASLD, Boston, USA	2016	28 hours
Half-yearly Meeting of the Dutch Association of Gastroenterology, Veldhoven	2017	12 hours
52nd International Liver Conference of EASL, Amsterdam	2017	28 hours
Monothematic conference, Cholangiocytes in health and disease, EASL, Oslo, Norway	2017	20 hours
Half-yearly Meeting of the Dutch Association of Hepatology, Veldhoven	2017	12 hours
The Liver Meeting, 68th Annual Meeting of the AASLD, Washington, USA	2017	28 hours
Half-yearly Meeting of the Dutch Association of Gastroenterology, Veldhoven	2018	12 hours
53rd International Liver Conference of the EASL, Paris, France	2018	28 hours

1. PhD training	Year	Workload
The XXV International Bile Acid Meeting: Bile Acids in Health and Disease, Dublin, Ireland	2018	18 hours
Half-yearly Meeting of the Dutch Association of Hepatology, Veldhoven	2018	12 hours
The Liver Meeting, 69th Annual Meeting of the AASLD, San Francisco, USA	2018	28 hours

Awards	
Young investigator bursary, International Liver Conference, EASL, Vienna, Austria	2015
Second best poster prize, Falk Symposium 197, Autoimmune Liver Diseases, Lisbon, Portugal	2015
International Travel Award, Liver Meeting, AASLD, San Francisco, USA	2015
Poster of Distinction, Liver Meeting, AASLD, Boston, USA	2016
International Travel Award, Liver Meeting, AASLD, Boston, USA	2016
Young investigator bursary, Monothematic Conference, EASL, Oslo, Norway	2017
International Travel Award, Liver Meeting, AASLD, Washington, USA	2017
Young investigator bursary, International Liver Conference, EASL, Paris, France	2018
Young Hepatologist Award for best Dutch hepatology paper, Dutch Association of Hepatology, Veldhoven, The Netherlands	2018

Attended seminars and workshops		
29th Erasmus Liver Day, Rotterdam	2014	6 hours
12th Post-AASLD symposium, Rotterdam	2014	2 hours
6e Lagerhuidsdebat , Utrecht	2014	3 hours
2nd 'PBC: past, present and future' meeting, Amsterdam	2015	6 hours
30th Erasmus Liver Day, Rotterdam	2015	6 hours
13th Post-AASLD symposium, Rotterdam	2015	2 hours
7e Lagerhuidsdebat , Utrecht	2015	3 hours
31th Erasmus Liver Day, Rotterdam	2016	6 hours
Symposium 31th NVH Anniversary, Rotterdam	2017	6 hours
PBC: how to move forward from here – symposium, Amsterdam	2017	8 hours
32th Erasmus Liver Day, Rotterdam	2017	6 hours

2. Teaching	Year
Lecturing	
Wetenschappelijk onderzoek bij PBC, landelijke bijeenkomst voor patiënten met PBC, Nederlandse Leverpatiënten Vereniging, Utrecht	2016
Fibrates in PBC, Early Morning Workshop, International Liver Conference, European Association of the Liver	2016
A case with itching, Erasmus Liver Day, Rotterdam	2016
Fibrates: who, what, where; Evening symposium cholestasis, Amsterdam	2017

Supervision	
Supervising graduation project Kiki Janssen, medical student, trends in liver transplantation in primary biliary cholangitis in Europe	2017

3. Extracurricular	
Board member Promeras, representing board of all PhD students, Erasmus MC, Rotterdam	'15-'16
PhD committee, Erasmus MC, Rotterdam	'15-'17
Promovendi Network Nederland (PNN)	'15-'17
Chair of Promeras, representing board of all PhD students, Erasmus MC, Rotterdam	'16-'17