Excellent outcome in patients with primary biliary cholangitis in Northwest Italy followed up for up to 30 years

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Objective Primary biliary cholangitis (PBC) is a rare chronic autoimmune cholangiopathy, characterized by a variable course and response to treatment. We aimed to describe long-term outcomes of PBC patients referred to three academic centres in Northwest Italy.

Methods This is an ambispective cohort study of PBC patients (retrospective component: diagnosis before 1 January 2019; prospective component: thereafter), including 302 patients: 101 (33%) followed up in Novara, 86 (28%) in Turin, 115 (38%) in Genoa. Clinical features at diagnosis, biochemical response to therapy and survival were analyzed.

Results Among the 302 patients (88% women, median age 55 years, median follow-up 75 months), alkaline phosphatase (ALP) levels significantly decreased during treatment with ursodeoxycholic acid (UDCA, $P < 0.0001$) and obeticholic acid ($P < 0.0001$). At multivariate analysis, ALP at diagnosis was predictive of 1-year biochemical response to UDCA [odds ratio 3.57, 95% confidence interval (CI) 1.4–9, $P < 0.001$]. Estimated median survival free of liver transplantation and hepatic complications was 30 years (95% CI 19–41). Bilirubin level at diagnosis was the only independent risk factor for the combined outcome of death, transplantation or hepatic decompensation (hazard ratio, 1.65, 95% CI 1.66–2.56, $P = 0.02$). Patients presenting with total bilirubin at diagnosis ≥0.6 times the upper normal limit (ULN) had a significantly lower 10-year survival compared to those with bilirubin <0.6 times ULN (63% vs. 97%, $P < 0.0001$).

Conclusion In PBC, both short-term response to UDCA and long-term survival can be predicted by simple conventional biomarkers of disease severity, obtained at diagnosis. Eur J Gastroenterol Hepatol 35: 899–906

Background

Primary biliary cholangitis (PBC) is a chronic autoimmune cholangiopathy characterized by slowly progressive damage to biliary ducts [1–4]. PBC is a rare disorder, with an estimated incidence of 1–2 cases/100.000/year and a reported prevalence varying between 1.9 and 40.2/100.000 [1,5,6]. It is more common among middle-aged women [2,7–9], with a female : male ratio close to 10 : 1 [5]. Recently, however, a change in PBC epidemiology has been described, with older age at diagnosis (median age from 46 years in the 1970s to 57 years from 2010 onward) and an increased prevalence [1,5–7,9–11]. An increase in the incidence of the disease in males has also been reported [11]. Although the disease usually has a mild and benign course, it may lead to severe chronic liver fibrosis, cirrhosis and liver failure needing liver transplantation [4]. It has been recently demonstrated that in Europe a worse clinical outcome is characteristic of the Northwest region in terms of rate of response, risk of decompensation, liver transplantation and death, while no difference has been described in age at diagnosis or sex according to geographical area [12].

Ursodeoxycholic acid (UDCA), the current first-line treatment for PBC [13], improves the prognosis of patients [14], although the risk of liver transplantation or liver-related death is not completely abolished. In patients who show an incomplete biochemical response or are intolerant to UDCA, second-line treatment with obeticholic acid (OCA) has shown improvement in cholestasis indexes [13].

A multiplicity of dynamic (i.e. related to treatment response) and static (i.e. obtained at diagnosis or at any time during disease course) risk stratification markers have been proposed [13]. Among the former, the main factors associated with a worse prognosis are young age at diagnosis, male sex, serum ALP, bilirubin levels and severity of liver disease according to liver stiffness measurement [15–17].

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In the present multicentric study, we aimed to describe the clinical and epidemiological profile of patients diagnosed with PBC and followed up in three tertiary liver centres in Northwest Italy (Piedmont and Liguria) and to assess the ability of biochemical endpoints to predict liver transplantation and liver-related complication-free survival.

Methods

Patients

We designed a multicentre, observational study, composed of both a retrospective and a prospective phase (ambispective). We included all patients diagnosed with PBC (diagnosis based on EASL diagnostic criteria) [13] and followed up at three Academic tertiary liver centres in Piedmont (Novara and Turin) and Liguria (Genoa).

The retrospective phase (until 31 December 2018) included patients with a previous diagnosis of PBC followed up at the participating units, while the prospective phase involved all patients with a new diagnosis of PBC after 1 January 2019. The last date of follow-up was 30 September 2020.

Cirrhosis was defined according to previous liver histology (when available), imaging (ultrasound or computed tomography or MRI) compatible with cirrhosis and clinical or laboratory data [18].

Exclusion criteria were lack of informed consent, overlap syndromes in which the main component was autoimmune hepatitis, and chronic cholangiopaties not fulfilling the diagnostic criteria for PBC [13].

The study was conducted in full accordance with Helsinki criteria and has been approved by our Ethical Committee (CE 75/20).

Data collection

Every included case was recorded in the web-based data capture software REDCap© (Research Electronic Data Capture) powered by the Vanderbilt University and partially supported by NIH (National Institute of Health), through the attribution of a univocal alphanumeric code for data pseudonymization. The detailed items that form the online database are reported separately.

Endpoints

The endpoints of interest for the study were death, liver transplantation and development of liver-related complications [ascites, variceal bleeding, hepatic encephalopathy and hepatocellular carcinoma (HCC)].

To assess liver transplantation-free survival and survival free from liver transplantation and liver decompensation, the time of follow-up was calculated for patients who died, were transplanted or developed liver-related complications at the time of death, liver transplantation or first complication, respectively, and for patients surviving without any endpoint of interest at the time of the last follow-up.

Biochemical response to UDCA was defined as serum ALP level <1.5 × upper normal limit (ULN) at 1 year of therapy according to Paris II criteria [19]. In addition, the secondary endpoint was the proportion of patients having ALP > 1.5 × ULN at diagnosis, at one and two years after the beginning of UDCA treatment, before and 1 year after add-on OCA treatment and at last follow-up.

Statistical analysis

Statistical analysis was performed by R 3.4.1 programming language (R Foundation for Statistical Computing, Vienna, Austria) together with package regression model strategies. Continuous variables are presented as median and interquartile range (IQR). Categorical data are summarized as absolute frequencies and relative proportions. Comparisons between groups were carried out using the Wilcoxon–Kruskal–Wallis test for numeric variables and Pearson's chi-square test for dichotomous variables.

Survival probabilities were calculated by computing Kaplan–Meier estimates; survival comparison between groups was carried out by the log-rank test. For long-term endpoints, a multivariable Cox regression model was built. The evaluation of endpoints during the time has been made by linear mixed effect models, with a random effect defined on patient ID. The statistical significance level was considered <0.05 (two-tailed).

Results

Patients

This study included 302 patients with PBC (267 females, female : male ratio 7.6 : 1), with a median age at diagnosis of 55 years (IQR, 48–64 years), 101 (33%) of whom followed up in Novara (diagnosis from 1995 to 2020), 86 (28%) in Turin (diagnosis from 1993 to 2020) and 115 (38%) in Genoa (from 1986 to 2020).

By stratifying patients upon time of diagnosis, 22 (7%) had been diagnosed from 1986 to 1999, 101 (33%) from 2000 to 2010, 143 (48%) from 2011 to 2018 and 36 (12%) from 2019 to 2020. Female : male ratio varied over time: 6.3 : 1 in 22 cases from 1986 to 1999, 5.7 : 1 in 101 patients diagnosed between 2000 and 2010 and 9.5 : 1 in 179 from 2010 to 2020.

A liver biopsy at diagnosis was available in 146 patients (48%): 33% presented at an early stage (Ludwig Stage I), 28% at an intermediate stage (stage II) and 39% presented at an advanced stage (stage III–IV).

The main features of our population at PBC diagnosis, subdivided by Centre, are reported in Table 1. Stratified for liver centre, the three groups were similar for prevalence of antimitochondrial antibodies (AMA) and antiglycoprotein 210 antibodies (anti-gp210) reactivity, proportion of patients with cirrhosis and its complications, liver stiffness value, serum levels of standardized aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), albumin, international normalized ratio (INR) and platelet count. The proportion of male patients resulted significantly higher in Genoa (female : male ratio of 4.2 : 1). In Novara, the proportion of patients with histological stage I–II was significantly higher compared to other centres ($P = 0.001$), and serum levels of ALP at diagnosis were significantly lower ($P = 0.04$). Conversely, serum bilirubin at baseline in patients from Turin resulted significantly higher ($P < 0.0001$).

Table 1. Comparison of main demographic and clinical features of population at primary biliary cholangitis diagnosis in the three participating centres

Feature	Novara (No. = 101)	Turin (No. = 86)	Genoa (No. = 115)	*P* value
Diagnosis 2019–2020, No.	19 (19%)	12 (14%)	5 (4%)	0.001
Females, No.	94 (93%)	78 (91%)	95 (83%)	0.01
Age, years	56 (49–67)	52 (43–62)	56 (51–63)	0.03
Age ≤ 45 years, No.	16 (16%)	28 (33%)	19 (16%)	0.009
ALP and GGT > 1.5 × ULN, No.	99 (98%)	86 (100%)	115 (100%)	0.08
AMA, No.	74 (75%)	71 (82%)	85 (74%)	0.25
Anti-Sp100, No.	15/96 (16%)	7/41 (17%)	1/45 (2%)	0.04
Anti-gp210, No.	9/96 (9%)	1/39 (3%)	3/45 (7%)	0.43
Liver biopsy, No.	47 (46%)	24 (28%)	75 (65%)	0.01
Ludwig 1–2 stage, No.	33/47 (70%)	8/24 (30%)	39/75 (52%)	0.001
Splenomegaly, No.	14 (14%)	7 (8%)	5 (4%)	0.02
Cirrhosis, No.	19 (19%)	12 (14%)	11 (10%)	0.10
Decompensated cirrhosis, No.	6 (6%)	5 (6%)	3 (3%)	0.32
Ascites, No.	3 (3%)	3 (4%)	3 (3%)	0.87
Variceal bleeding, No.	2 (2%)	0	0	0.08
Hepatic encefalopathy, No.	0	0	1 (1%)	0.26
Hepatocellular carcinoma, No	1 (1%)	0	1 (1%)	0.94
Liver stiffness (LS), kPa	7.2 (4.8–9.1)	6.5 (5.1–9.1)	7.4 (6.1–10.5)	0.57
AST, ×ULN	1.1 (0.8–1.7)	0.9 (0.7–1.6)	1.2 (0.9–1.7)	0.37
ALT, ×ULN	1.2 (0.8–2.1)	1.2 (0.7–2.2)	1.4 (0.9–1.9)	0.80
GGT, ×ULN	3.4 (1.8–5.7)	3.9 (2.2–5.9)	4.7 (2.1–7.5)	0.54
ALP, ×ULN	1.4 (0.8–2.4)	1.9 (2.2–2.9)	2.0 (1.2–3.2)	0.04
Total bilirubin, mg/dl	0.6 (0.5–1)	1.1 (0.7–1.5)	0.6 (0.4–0.8)	<0.0001
Albumin, g/dl	4.2 (3.9–4.4)	3.9 (3.6–4)	4.1 (3.9–4.3)	0.08
INR	1 (0.94–1.05)	1.02 (0.97–1.17)	0.98 (0.95–1.07)	0.25
Platelets, $\times 10^9$/l	235 (183–289)	197 (110–316)	230 (199–274)	0.49

Categorical variables are reported as absolute frequency (proportion), continuous variables as median (interquartile range).
ALT, alkaline phosphatise; AMA, antimitochondrial antibodies; Anti-gp210, antiglycoprotein 210 antibodies; Anti-Sp100, nuclear sp100 antibodies; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; INR, international normalized ratio; ULN, upper normal limit.

The proportion of new diagnoses (since 2019) resulted significantly higher in Novara and the age at diagnosis was significantly lower in Turin. The prevalence of nuclear sp100 antibodies (anti-Sp100) autoantibodies was significantly lower in Genoa ($P = 0.03$).

By comparing patients with PBC diagnosis before and after 2019, female proportion and age at diagnosis were similar. Autoantibodies reactivity (AMA, anti-Sp100, anti-gp210) resulted 97% among those with diagnosis since 2019 and 87% in the retrospective cohort ($P = 0.07$), with a rate of anti-Sp100 reactivity significantly lower in the latter (33% vs. 9%, $P < 0.0001$) (Table 2).

Patients who received a diagnosis since 2019 had a higher rate of cirrhosis, liver decompensation and splenomegaly, as well as significantly higher INR. The two groups were not significantly different regarding serum levels of AST, ALT, GGT, ALP, bilirubin, albumin, platelet count and baseline liver stiffness.

Biochemical response to treatment

All patients received UDCA as first-line treatment. Table 3 reports the features of liver biochemistry at diagnosis, after 1 and 2 years of UDCA treatment and at the last follow-up visit. During UDCA therapy, serum ALP level and the proportion of patients with ALP > 1.5 × ULN decreased significantly ($P < 0.0001$). Liver stiffness values also decreased ($P = 0.03$). At univariate analysis, predictive factors of biochemical response to UDCA at 1 year were ALT, GGT and ALP × ULN ($P < 0.0001$). At multivariate analysis, serum ALP level at diagnosis was the only factor independently associated with biochemical response after 1 year of UDCA treatment [odds ratio (OR) 3.57, 95% confidence interval (CI) 1.4–9, $P < 0.001$].

Liver stiffness at diagnosis was not significantly associated with biochemical response at 1 year of treatment ($P = 0.08$).

Among the 302 included patients, 36 underwent second-line therapy with add-on OCA to UDCA. OCA was prescribed following the Italian National Health Service reimbursability criteria, that is, ALP ≥ 1.5 × ULN and bilirubin ≤2 mg/dl after at least 12 months of treatment with UDCA, or the intolerance to UDCA. During the first year of OCA treatment, the proportion of patients with ALP > 1.5 × ULN (from 88 to 50%, $P = 0.01$) and median serum ALP levels (2.2 × ULN before OCA and 1.6 × ULN at 1 year of treatment, $P < 0.0001$) significantly decreased.

Predictive factors for achieving ALP < 1.5 × ULN at last follow-up by Cox multivariate analysis were serum ALP and ALT levels at diagnosis ($P = 0.03$ both). The higher the level of ALP and ALT at diagnosis, the lower the probability of achieving ALP < 1.5 × ULN at the end of follow-up.

By using a time-dependent model the probability of ALP ≥ 1.5 × ULN was significantly reduced by UDCA (–56% at 1 year of treatment, –59% at 2 years and at last follow-up, $P < 0.001$) and by UDCA plus OCA (–74% at 1 year of treatment, $P < 0.001$) (Fig. 1).

None of the 302 patients underwent treatment with fenofibrate due to the absence of reimbursability by the Italian National Health System and their prescription as an off-label therapy for PBC.

Outcome

During a 2472 person-year follow-up (median of 75 months, range 6–402, IQR 28–150 months), four patients (1.3%) died (two men and two women), one

of whom due to HCC, two due to pneumonia (one of whom while in waiting list for liver transplantation due to HCC), one for acute cardiovascular event. Five patients (1.3%) underwent liver transplantation (four women and one man): one for untreatable pruritus, two for decompensated cirrhosis and two for HCC. Incidence rate of death and liver transplantation was three per 1000 person-year.

Overall, 23 patients (8%) developed complications of cirrhosis: 16 ascites, 2 hepatic encephalopathy, 4 variceal bleeding, 4 HCC; 2 patients developed more than one complication. Moreover, seven patients (2%) developed severe portal hypertension (high-risk oesophageal varices treated with endoscopic band ligation), without acute bleeding.

At Kaplan–Meier analysis, liver transplantation-free survival probability was 99% at 1 year and 98%, 95%, 94% and 92% at 5, 10, 15 and 20 years, respectively (Fig. 2a); estimated median survival free of liver transplantation and liver complications was 30 years (95% CI, 19–41) (Fig. 2b). Liver transplantation and liver complications-free survival probability was 99% at 1 year and 96%, 92%, 86% and 78% at 5, 10, 15 and 20 years, respectively (Fig. 2b).

Predictors of outcome

At the multivariate Cox regression analysis (Table 4), the only independent predictor of the combined outcome of death, liver transplantation or development of liver complications was total serum bilirubin levels at diagnosis (hazard ratio 1.65, 95% CI 1.66–2.56, $P = 0.02$). For an increase in baseline bilirubin of a quartile (from 0.4 to 0.8 × ULN) the risk of death, liver transplantation and

Table 2. Comparison between main demographic and clinical features of 302 primary biliary cholangitis patients at diagnosis, stratified for time of diagnosis until 2018 and new diagnosis 2019–2020

Feature	Diagnosis until 2018 (No. = 266)	Diagnosis 2019–2020 (No. = 36)	*P* value
Females, No.	234 (88%)	33 (92%)	0.51
Age, years	55 (49–63)	56 (47–69)	0.49
ALP and GGT > 1.5 × ULN, No.	263 (99%)	36 (100%)	1.0
AMA, No.	198 (74%)	32 (89%)	0.18
Anti-Sp100, No.	14 (5%)	9 (25%)	<0.0001
Anti-gp210, No.	10 (4%)	3 (8%)	0.36
Liver biopsy, No.	134 (50%)	12 (33%)	0.19
Ludwig 1–2 stage, No.	74/134 (55%)	6/12 (50%)	0.89
Splenomegaly, No.	19 (7%)	7 (19%)	0.02
Cirrhosis, No.	32 (13%)	10 (30%)	0.01
Decompensated cirrhosis, No.	10 (4%)	4 (12%)	0.05
Ascites, No.	7 (3%)	2 (6%)	0.33
Variceal bleeding, No.	1 (0.3%)	1 (3%)	0.10
Hepatic encefalopathy, No.	1 (0.3%)	0	0.71
Hepatocellular carcinoma, No (%)	2 (1%)	0	0.6
Liver stiffness, kPa	7.2 (5.4–8.9)	6.4 (5–10.1)	0.91
AST, ×ULN	1.1 (0.8–1.6)	1.3 (1–1.9)	0.09
ALT, ×ULN	1.2 (0.8–1.98)	1.4 (0.9–2)	0.38
GGT, ×ULN	3.3 (2–6.4)	4.1 (2.1–7)	0.5
ALP, ×ULN	1.7 (0.9–2.8)	1.5 (1–3.1)	0.76
Total bilirubin, mg/dl	0.7 (0.5–1)	0.7 (0.6–1.7)	0.07
Albumin, g/dl	4.1 (3.9–4.4)	4.1 (3.9–4.4)	0.63
INR	0.99 (0.94–1.05)	1.04 (1–1.08)	0.03
Platelets, $\times 10^9$/l	227 (180–285)	246 (199–297)	0.34
Immunologic comorbidities, No.	88 (33%)	9 (25%)	0.34
Sjögren's syndrome	8 (3%)	1 (3%)	0.94
Autoimmune thyroid disease	43 (16%)	2 (6%)	0.09
Systemic sclerosis	19 (7%)	4 (11%)	0.40
Rheumatoid arthritis	5 (2%)	0	0.41
Other	29 (11%)	3 (8%)	0.70

Categorical variables are reported as absolute frequency (proportion), continuous variables as median (interquartile range).
ALP, alkaline phosphatise; ALT, alanine aminotransferase; AMA, antimitochondrial antibodies; Anti-gp210, antiglycoprotein 210 antibodies; Anti-Sp100, nuclear sp100 antibodies; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; INR, international normalized ratio; ULN, upper normal limit.

Table 3. Clinical features at diagnosis and follow-up of 302 primary biliary cholangitis patients

Features	At diagnosis No. = 302	After 1 year of UDCA therapy No. = 150	After 2 years of UDCA therapy No. = 118	At last follow-up No. = 302	*P* value
Liver stiffness, kPa	7.1 (5.2–9)	6.4 (5.1–8.4)	5.6 (4.1–7.7)	6 (4.6–8.1)	0.03
ALP > 1.5 × ULN	55%	24%	21%	24%	<0.001
ALP, ×ULN	1.6 (0.9–2.8)	1 (0.7–1.5)	0.9 (0.7–1.3)	1 (0.7–1.5)	<0.001
Bilirubin, mg/dl	0.7 (0.5–6.8)	0.6 (0.4–0.9)	0.6 (0.4–0.8)	0.6 (0.3–0.9)	0.24
Albumin, g/dl	4.1 (3.9–4.4)	4.2 (3.9–4.4)	4.2 (4–4.5)	4.1 (3.9–4.4)	0.29
Platelets, $\times 10^9$/ml	240 (192–300)	259 (211–300)	258 (193–300)	234 (189–207)	0.46

Categorical variables are reported as absolute frequency (proportion), continuous variables as median (interquartile range).
ALP, alkaline phosphatise; UDCA, ursodeoxycholic acid; ULN, upper normal limit.

liver complications during the follow-up increased by 65%.

By stratifying 143 patients with available total bilirubin levels at diagnosis (<0.6 × ULN vs. ≥0.6 × ULN), liver transplantation-free survival and liver transplantation and liver complication-free survival were significantly lower among patients with total bilirubin ≥0.6 × ULN at diagnosis ($P = 0.01$ and $P < 0.0001$, respectively) (Fig. 3a and b).

The 10-year liver transplantation-free survival probability was 100% among patients with total bilirubin <0.6 × ULN at diagnosis, whereas it was 81% among patients with total bilirubin ≥0.6 × ULN at diagnosis ($P = 0.01$).

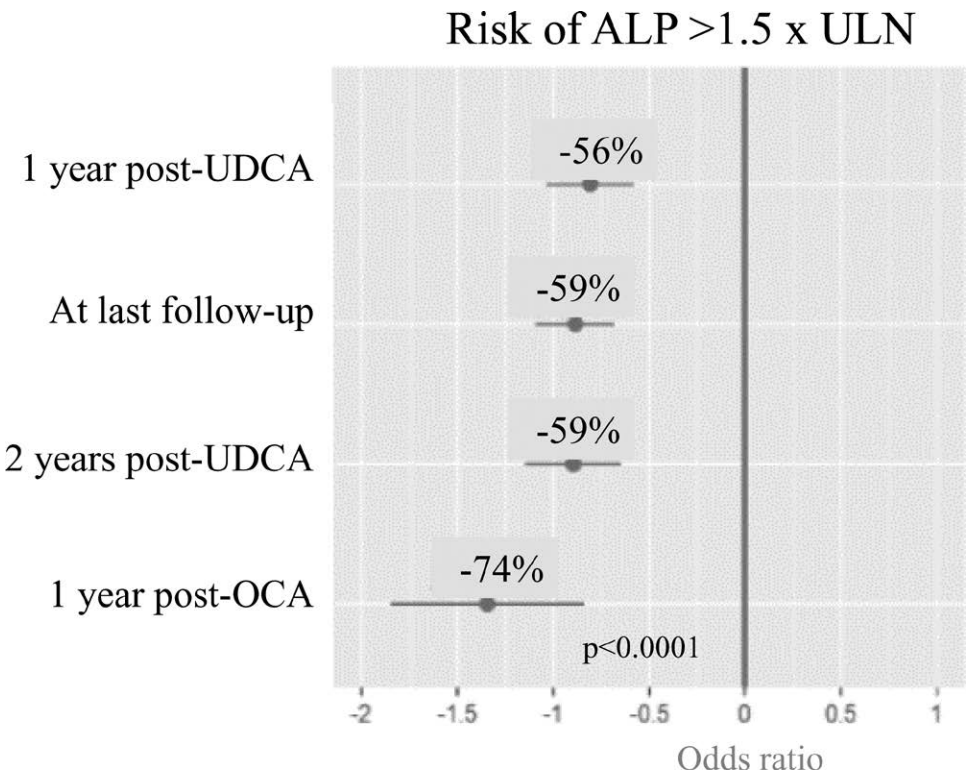

Fig. 1. Probability of having ALP ≥ 1.5 × ULN during treatment with UDCA and OCA by linear mixed effect model. OCA, obeticholic acid; UDCA, ursodeoxycholic acid; ULN, upper normal limit.

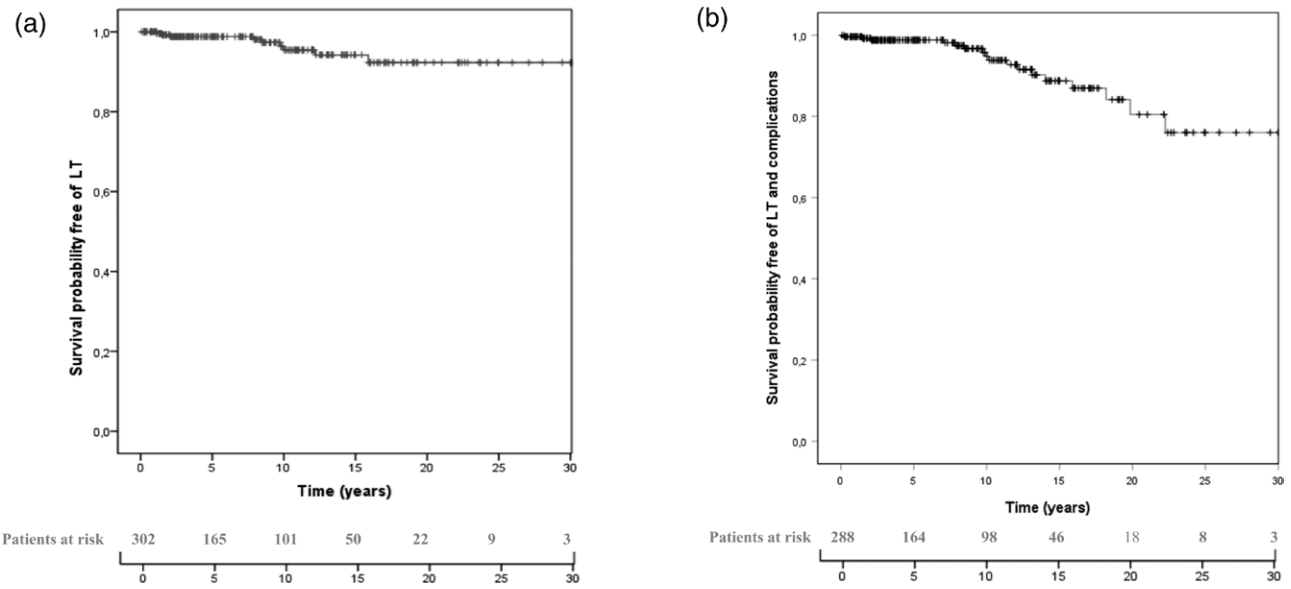

Fig. 2. (a) Liver transplantation-free survival probability in 302 PBC patients and (b) liver transplantation and complications-free survival probability in 288 PBC patients (excluding 14 patients with complications at diagnosis). PBC, primary biliary cholangitis.

Finally, the 10-year liver transplantation- and liver complications-free survival probability was 97% among patients with total bilirubin <0.6 × ULN at diagnosis vs. 63% among those with bilirubin ≥0.6 × ULN ($P < 0.0001$).

Discussion

The present multicentric observational study assessed the clinical profile of 302 PBC patients attending three tertiary academic liver centres in Northwest Italy (Piedmont and Liguria regions) during a median follow-up of 75 months, with 10% of patients followed up for more than 30 years. Among these patients, median age at PBC diagnosis was 55 years, in line with the data reported in large studies [1,6,7,9,12]. By stratifying the patients according to the period of diagnosis a significant increase in median age was observed: 48 years in the period 1986–1999, 55 years for patients diagnosed between 2000 and 2010 and 57 years in the group 2011–2020 ($P < 0.0001$). This finding fully confirms what is reported in a large, international study including 4800 patients who received a diagnosis of PBC between 1970 and 2014 in 17 centres in Europe and Northern America: the authors described a progressive increase in median age at diagnosis, from 46.9 in the 1970s to 57 from 2010, with a significant change in age distribution at diagnosis [10].

Overall, in our patients the female : male ratio was 7.6 : 1, in line with that reported by others, ranging from 8 to 10 : 1 [1,11]. The most recent studies, however, have reported an increasing trend in male prevalence. As an example, the epidemiologic study by Lleo *et al*. [7], based on administrative data in Denmark and Lombardy, highlighted a trend towards the reduction of female : male ratio to 2.3 : 1 in Lombardy and 4 : 1 in Denmark. Similar data were reported in the study by Lu *et al*., which included 3400 patients in the US [9] with a female : male ratio of 3.9 : 1. In our study, the lowest female : male ratio was observed among patients attending the Liver Clinic in Genoa, that displayed a female : male ratio 4.2 : 1, comparable to that reported in Denmark [7] and USA [9].

Moreover, by stratifying our patients according to period of diagnosis we found that female : male ratio varied over time: 6.3 : 1 in 22 cases from 1986 to 1999, 5.7 : 1 in 101 patients diagnosed between 2000 and 2010 and 9.5 : 1 in 179 from 2010 to 2020. These data suggest a trend of new increase female : male ratio during the last decade, towards a value which has been widely reported in the past.

From January 2019 to September 2020, 36 new diagnoses of PBC have been made in the three tertiary liver centres, representing 12% of our total population: 19 new diagnoses were made in Novara (corrected for resident population, it corresponds to an estimated incidence rate of 11 per 100 000 person-years), 12 in Turin (corresponding to an estimated incidence of three per 100 000 person-years) and five in Genoa (corresponding to an estimated incidence of 0.6 per 100 000 person-years). These findings of the relatively high number of new cases

Table 4. Multivariate Cox regression analysis of risk factors associated with death, liver transplantation and liver complications

	Multivariate Cox regression analysis	
	Hazard ratio (95% CI)	*P* value
ALP × ULN	0.98 (0.29–3.31)	0.97
Total bilirubin × ULN	**1.65 (1.66–2.56)**	**0.02**
AST × ULN	2.03 (0.21–19.0)	0.53
ALT × ULN	0.04 (0.01–0.11)	0.11

ALP, alkaline phosphatise; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; ULN, upper normal limit.
In the univariate analysis were included the same variables as in the multivariate analysis.

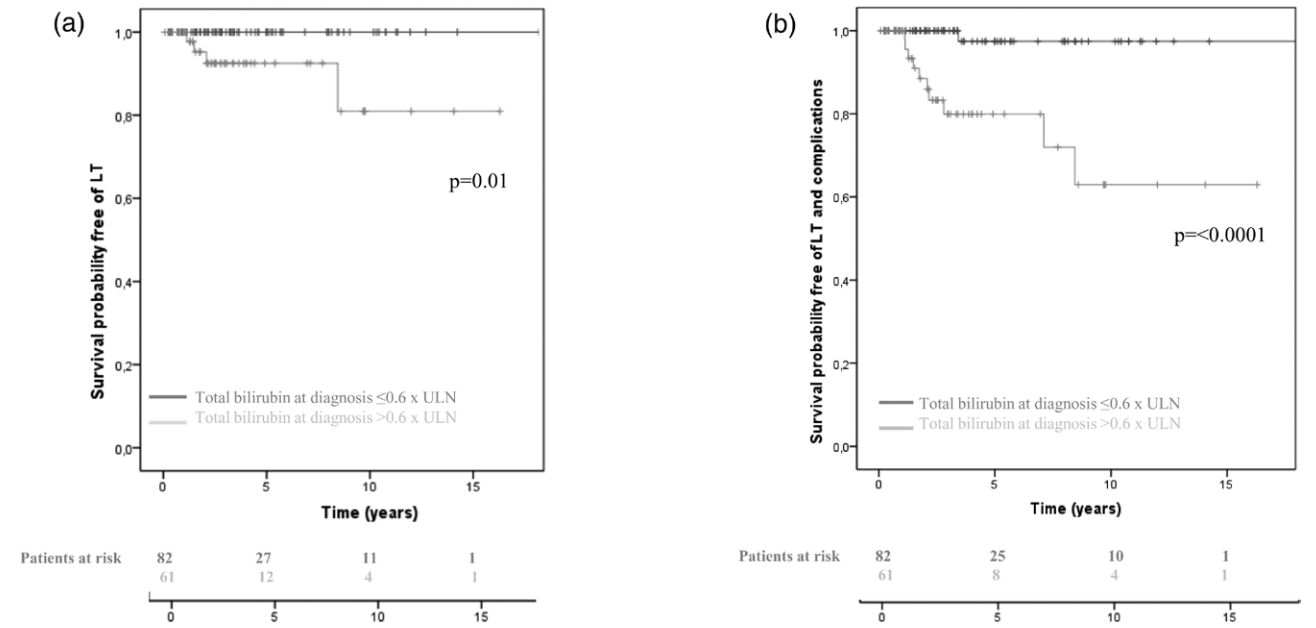

Fig. 3. (a) Liver transplantation-free survival probability and liver transplantation and (b) complications-free survival probability in 302 patients with PBC, according to total bilirubin serum levels at diagnosis (≤0.6 × ULN vs. >0.6 × ULN). PBC, primary biliary cholangitis; ULN, upper normal limit.

in Novara might be explained by an increased referral to our Centre of patients previously referred elsewhere. In addition, refinement of diagnostic techniques, particularly serology, may have played a role. In fact, antinuclear antibodies (ANA)-specific anti-Sp100 and anti-gp210 are assuming a relevant diagnostic role. In our study, autoantibodies including AMA and ANA-specific were positive in 87% of cases diagnosed until 2018 vs. 97% in 2019–2020, with a significant increase in the proportion of anti-Sp100 reactivity ($P < 0.0001$). Furthermore, we observed a significantly higher proportion of cirrhotic patients in the prospective cohort, maybe due to a relatively small sample size of patients. This data needs further investigation in larger prospective cohorts.

Regarding biochemical response to UDCA treatment, serum ALP value at diagnosis was the only significant predictor of response, with an OR higher than 3. By assessing the dynamics of ALP levels during follow-up, we showed an increased probability of obtaining a biochemical response to UDCA according to duration of treatment. By using a time-dependent model, we could demonstrate that both UDCA and UDCA plus OCA significantly reduced the probability of having ALP ≥ 1.5 × ULN over time (–56% of probability after 1 year of UDCA treatment, –74% after 1 year of OCA treatment). This confirms what has been already described in the literature, supporting the guideline indication of maintaining lifelong UDCA treatment [13,20].

One major finding of the present study is the demonstration of excellent long-term survival, in agreement with what was demonstrated among patients belonging to the Southeast region of Europe [12]. Early diagnosis and start of UDCA treatment are both proved to be significantly associated with better outcomes, that is, increased median survival [21], which is about 80–85% at 20 years among UDCA-treated patients who achieve biochemical response [22]. We confirmed these data in our patients who showed a 20-year liver transplantation-free survival of 92%. This excellent survival is partly due to the high proportion of patients diagnosed at an early stage: less than 20% of patients, in fact, were cirrhotic at diagnosis. This is in line with what has been previously reported by Murillo Perez, who found that diagnosis at an early stage increased from 41% in the 1970s to 72% in the 1990s [10].

The value of bilirubin at diagnosis has been confirmed in our patient population as the only independent predictor of risk of death, liver transplantation and liver-related complications. The strong predictive role of bilirubin in PBC is well known since 1979 [23] and has been widely confirmed in other studies. Among them, the study by Lammers *et al*. documented in 4845 patients that total bilirubin higher than the normal value at 1-year follow-up was predictive of unfavourable survival prognosis [24]. In fact, bilirubin value is included in every predictive model of UDCA response [19,20,25–29]. A recent multicentric study involving 2281 patients showed that the risk of death and transplantation among PBC patients was significantly increased also with bilirubin levels within the normal range, if higher than 0.6 × ULN [16]. By stratifying our patients according to serum total bilirubin at diagnosis, we fully confirmed the finding reported by Murillo Perez *et al*.: patients with bilirubin ≥0.6 × ULN at diagnosis had a 10-year survival significantly lower compared to those with total bilirubin <0.6 × ULN at diagnosis (63% vs. 97%, $P < 0.0001$).

Besides bilirubin, liver stiffness has recently emerged as a major, independent, validated predictor of PBC outcome: the lower the liver stiffness, the higher the survival time without poor clinical outcomes in PBC patients has been demonstrated [17]. Among 125 patients with available baseline liver stiffness measurement, liver transplantation-free survival resulted significantly higher in those with baseline liver stiffness < 10 kPa, confirming the prognostic role of liver stiffness reported in larger multicentric studies. We look forward to covering this analysis in a larger prospective cohort of patients, for whom baseline and follow-up liver stiffness values will be fully available.

In conclusion, among the 302 PBC patients from Piedmont and Liguria, we confirm the clinical and epidemiological profile of PBC reported in other large studies, as well as the value of bilirubin as the strongest predictor of survival.

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Conflicts of interest

There are no conflicts of interest.

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