

# Biochemical criteria at 1 year are not robust indicators of response to ursodeoxycholic acid in early primary biliary cirrhosis: results from a 29-year cohort study

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## SUMMARY

### Background

In primary biliary cirrhosis (PBC), biochemical criteria at 1 year are considered surrogates of response to ursodeoxycholic acid (UDCA). However, due to the slow natural history of PBC, evaluation at 1 year may be suboptimal to assess the therapeutic response, particularly in early disease.

### Aim

To determine whether evaluation of biochemical criteria at 1 year is a reliable surrogate of UDCA response in early PBC.

### Methods

We analysed the prospectively collected data of 215 patients (untreated = 129; UDCA-treated = 86) with early PBC (normal baseline bilirubin/albumin) and a median follow-up of 8 years (range: 1–29.1). The 1-year attainment rates of the Barcelona, Paris-I, Paris-II and Toronto definitions, and their predictive relevance for a poor outcome (death, transplantation, complications of cirrhosis), were assessed either as a result of UDCA or no treatment. Independent associations with attaining each UDCA response definition were identified by multivariate analysis.

### Results

Untreated patients displayed 1-year biochemical features compatible with 'treatment response' at rates (Barcelona: 36.4%, Paris-I: 66.7%, Toronto: 59.7%, Paris-II: 40.3%) similar to those obtained under UDCA. Depending on the definition, baseline  $ALP \leq 3 \times ULN$  (OR: 4.80–35.90),  $AST \leq 2 \times ULN$  (OR: 5.63–9.34) and early histological stage (OR: 3.67–3.87) were the stronger predictors for attaining the criteria. UDCA treatment was associated with attaining Barcelona (OR = 2.16) and Paris-II (OR = 2.84), but not Paris-I, and not Toronto definition when excluding late histological cases. Paris-I criteria were significantly predictive of long-term outcomes (HR = 2.83) in untreated patients.

### Conclusions

In early PBC, biochemical criteria at 1 year reflect severity of the disease rather than the therapeutic response to UDCA.

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## INTRODUCTION

Primary biliary cirrhosis (PBC) results from progressive immune-mediated destruction of small and medium size intrahepatic bile ducts, which may lead to cirrhosis, liver failure and requirement for liver transplantation (LT).<sup>1</sup> Currently, ursodeoxycholic acid (UDCA) is the only approved therapy in PBC,<sup>2–5</sup> which improves liver biochemistry,<sup>6, 7</sup> and may delay histological progression,<sup>8, 9</sup> although an overall survival benefit is disputed.<sup>10, 11</sup> Nonetheless, the patients' transplant-free survival with UDCA remains lower than in age- and sex-matched controls from the general population; thus, new therapeutic approaches are needed.<sup>12</sup> As the natural history of PBC is slow, definition of surrogate markers of disease progression is of cardinal importance for both clinical practice and design of clinical trials.<sup>13</sup> Recently, a number of cohort studies have shown a correlation between the magnitude of biochemical response to UDCA and long-term outcomes, including transplantation-free survival<sup>14–16</sup> and histological progression.<sup>17</sup> Thus, biochemical responses evaluated after 1 or 2 years of UDCA therapy have become surrogate markers of therapeutic efficacy in PBC. However, a point for criticism may be that no study proposing an UDCA biochemical response has so far included untreated patients with PBC.<sup>14–18</sup> Indeed, the development of UDCA response criteria relied on separating 'responders' to UDCA with favourable clinical outcomes from 'nonresponders', compared with age- and sex-matched healthy controls, but had no comparison with matched untreated PBC patients. Due to the lack of this measure, the strength of the causal association between biochemical responses and the actions of UDCA might have been not thoroughly assessed. Notably, in a recent abstract, the most validated UDCA response criteria, Paris-I (PA-I), were shown to maintain excellent prognostic ability irrespective of UDCA therapy, questioning their ability to reflect a drug effect.<sup>19</sup> Currently, most PBC patients are diagnosed early during their disease course, when bilirubin is normal (and thus impossible to be evaluated prognostically), whereas alkaline phosphatase (ALP) and aspartate transaminase (AST) may be minimally elevated. Thus, early evaluation of these variables with respect to assessing a therapeutic response may be problematic. Not surprisingly, the most validated biochemical response criteria [Barcelona (BA) and PA-I] do not predict outcomes with UDCA in early PBC,<sup>16, 18</sup> whereas the Rotterdam criteria<sup>16</sup> have little applicability because they require abnormal baseline bilirubin and albumin values. Newer criteria [Paris-II (PA-II)] are now proposed as best in early PBC,<sup>18</sup> but

still lack external validation. Focusing on early PBC, the present study aimed to investigate the strength of the causal association between treatment with UDCA and four published biochemical response criteria evaluated at 1 year. Using a cohort of untreated early PBC patients with prolonged follow-up, we determined to which extent 1-year UDCA response definitions may occur, and be predictive of poor prognosis, in patients receiving no treatment. Magnitude of this effect was compared with that obtained under UDCA in our institution and published series. Furthermore, we identified independent associations with attaining each biochemical definition, including 1-year treatment with UDCA as a candidate variable.

## METHODS

### Patients

In a prospectively collected database of consecutive patients with PBC referred between 1977 and 2001, used in earlier publications,<sup>20–23</sup> we evaluated those with non-histological definition of early disease, i.e. normal baseline bilirubin and albumin levels.<sup>12</sup> The diagnosis of PBC was made with positive anti-mitochondrial antibodies (AMA) and ALP > 1.5x the upper normal limit (ULN), and/or by liver histology compatible with PBC. Overall, there were 81 (37.7%) patients with baseline ALP ≤ 1.5x ULN (all AMA-positive), whereas another 25 (11.6%) were AMA-negative. In both these subsets of patients, the diagnosis of PBC was established histologically. Liver biopsies were evaluated if performed within 12 months of database entry and staged using Scheuer's classification.<sup>24</sup> All patients had follow-up data including UDCA biochemical response parameters (serum bilirubin, ALP and AST concentrations), before and after 1 year from presentation and/or diagnosis, and/or UDCA starting date. Data-points were 3- to 6-monthly routine clinical visits, or whenever attending or admitted to hospital. The date of diagnosis, presentation to Royal Free Hospital, UDCA starting date, date of complications, date of last follow-up, date of LT or death and causes of death were recorded. Nontreatment with UDCA was either because patients were diagnosed and followed up in the pre-UDCA era, or because this was the choice made following consultation between the patient and the treating physician, who was a hepatologist in all cases. We analysed untreated patients from when first seen at the Royal Free Hospital, and treated patients from first date of UDCA administration. Autoimmune overlap syndrome<sup>25</sup> or other concomitant liver disease, immunosuppressive

drug use and follow-up <1 year were exclusion criteria. As late histology adversely impacts both UDCA response and long-term prognosis, we planned a subanalysis, excluding patients with Scheuer's stages III/IV at baseline.

### Definition of biochemical and clinical endpoints

Biochemical features in untreated and treated PBC patients were evaluated 1 year after study entry, with regard to fulfilling four published UDCA response definitions (Table 1). Each biochemical definition, irrespective of attended or not as a result of UDCA, was evaluated as a prognostic surrogate for an adverse outcome, defined as whichever occurred first of complications of cirrhosis (ascites, variceal bleeding, hepatic encephalopathy or hepatocellular carcinoma), liver-related death (liver failure and/or complications above) or LT. Follow-up was censored at death or LT or at first occurrence of complication, or at last follow-up. The Toronto criteria<sup>17</sup> were based on 2 years of UDCA therapy, using a histological endpoint: ALP  $\leq 1.67 \times$  ULN with a one-stage increase or ALP  $\leq 1.76 \times$  ULN with a two-stage increase. To make our results comparable to a recent study in early PBC,<sup>18</sup> we used the ALP  $< 1.76 \times$  ULN TO criteria with a clinical endpoint. We did not evaluate the Rotterdam definition,<sup>16</sup> as it was not applicable to our patients with normal baseline bilirubin and albumin.

### Statistical analysis

The baseline demographic and clinical characteristics are presented as percentage, or means with standard

deviations (s.d.), or median (range); comparisons were performed with  $\chi^2$  or Fisher's exact tests for categorical data and Student's *t* or Mann–Whitney *U* tests for continuous data, as appropriate. Comparisons of biochemical values before and after 1 year were performed using the Wilcoxon signed-rank test. Using the whole cohort ( $n = 215$ ), univariate analysis was evaluated to identify associations with attaining 1-year response criteria defined by BA, PA-I, TO and PA-II criteria. Variables achieved significance ( $P < 0.1$ ) in univariate analysis, entered into multivariate logistic regression, except for UDCA therapy, which was kept independent of its univariate significance. Long-term outcomes were estimated with Cox proportional-hazards regression models using the average hazard ratio (HR) with 95% confidence intervals (CI). Time-to-event analysis was evaluated by Kaplan–Meier method and comparisons were evaluated using log-rank testing. The statistical analyses were performed using SPSS statistics version 19 (IBM, Armonk, NY, USA). All analyses were two-sided and  $P$ -values  $< 0.05$  were considered statistically significant.

## RESULTS

### Descriptive data

From the 498 consecutive patients with PBC, 228 fulfilled nonhistological definition of early disease. Thirteen patients were excluded: 11 had incomplete biochemical data, and in 2, follow-up was <1 year. Therefore, 215 patients (untreated = 129; UDCA-treated = 86, 96.3% females, age:  $56.8 \pm 11.8$  years) finally comprised the study population (Table S1). In those receiving UDCA, the mean dosage was 15 mg/kg/day (s.d. = 5; range 7–23 mg/kg/day): 34 received low-dose UDCA and 52 received high-/standard-dose UDCA (mean 17 mg/kg/day; range 13–23). No significant baseline clinical or biochemical differences were detected between standard-/high-dose and low-dose UDCA groups (data not shown). The untreated and UDCA-treated groups had similar demographic, clinical and biochemical characteristics (Table 2), except for median baseline bilirubin (untreated: 9; 3–16  $\mu\text{mol/L}$ , UDCA-treated: 10; 5–17  $\mu\text{mol/L}$ ,  $P = 0.04$ ). In patients with liver biopsy (untreated: 85; 65.9%, UDCA-treated: 77; 89.5%), late histological stages (Scheuer's stages III/IV) were found in 25.9% untreated vs. 10.4% UDCA-treated patients ( $P = 0.01$ ). Mean time between diagnosis and presentation to Royal Free Hospital was  $1.7 \pm 2.1$  years, whereas between diagnosis and starting of UDCA was  $2.1 \pm 3.5$  years ( $P = 0.060$ ), obviating lead-time bias in

**Table 1** | Published criteria of biochemical response to ursodeoxycholic acid in patients with primary biliary cirrhosis

Criterion	Definition of biochemical response
Barcelona <sup>15</sup>	ALP decrease >40% from baseline or to normal after 1 year of UDCA.
Paris-I <sup>14</sup>	ALP $\leq 3 \times$ ULN, AST $\leq 2 \times$ ULN and normal bilirubin after 1 year of UDCA.
Toronto <sup>17</sup>	ALP $\leq 1.67 \times$ ULN* or ALP $\leq 1.76 \uparrow$ after 2 years of UDCA.
Paris-II <sup>18</sup>	ALP and AST $\leq 1.5 \times$ ULN with normal bilirubin after 1 year of UDCA.

ALP, alkaline phosphatase; UDCA, ursodeoxycholic acid; AST, aspartate transaminase; ULN, upper limit of normal.

Toronto criteria were developed using a histological endpoint.

\* Defining nonresponse as a one-stage increase.

† Defining nonresponse as a two-stage increase.

**Table 2 | Comparison of baseline characteristics between patients with early primary biliary cirrhosis (normal baseline bilirubin and albumin) receiving no treatment and those treated with ursodeoxycholic acid**

	Untreated (n = 129)	UDCA-treated (n = 86)
Age	56.8 (11.4)	56.9 (12.4)
Female gender	125 (96.9)	82 (95.3)
Total bilirubin (µmol/L)	9 (3–16)*	10 (5–17)*
ALP (U/L)	240 (50–1730)	226.5 (42–1074)
AST (U/L)	44.5 (18–312)	58 (14–270)
Albumin (g/L)	42 (35–51)	42 (35–57)
Prothrombin time (s)	12.9 (0.9)	12.1 (1.9)
INR	0.95 (0.1)	0.99 (0.07)
AMA-positive	117 (90.7)	73 (84.9)
IgM (g/L)	3.7 (3.2)	4.1 (3.6)
IgG (g/L)	15.5 (11.5)	15.4 (6.5)
Histology staging (n=162)		
	n = 85	n = 77
I	51 (60)	33 (42.9)
II	12 (14.1)	36 (46.8)
III	15 (17.6)	5 (6.5)
IV	7 (8.2)	3 (3.9)
Late (III, IV)	22 (25.9)**	8 (10.4)**
Risk scores†		
Mayo risk score <sup>27</sup>	3.99 (0.63)	3.99 (0.71)
Royal Free score <sup>20</sup>	−4.73 (0.82)	−4.53(0.91)

ALP, alkaline phosphatase; AST, aspartate transaminase; INR, international normalised ratio; AMA, anti-mitochondrial antibody; IgM, immunoglobulin M; IgG, immunoglobulin G. Qualitative variables are reported as n(%); quantitative variables as mean (±standard deviation), except for bilirubin, ALP and AST and albumin reported as median (range). Statistically significant differences between the untreated and UDCA-treated cohorts are indicated by asterisks: \**P* = 0.04, \*\**P* = 0.01.

† Risk scores were calculated as follows: Mayo Risk Score,  $R = 0.871 \times \log_e(\text{bilirubin [mg/dL]}) - 2.53 \times \log_e(\text{albumin [g/dL]}) + 0.039 \times \text{age (years)} + 2.38 \times \log_e(\text{prothrombin time[s]}) + 0.859 \times \text{oedema (0 = no oedema, no diuretic therapy; 0.5 = oedema, no diuretic therapy or no oedema, diuretic therapy; 1 = oedema and diuretic therapy)}$ ; Royal Free Score,  $R = [0.55 \times \text{age (years)} + 21 \times \log_{10}(\text{bilirubin [µmol/L]}) - 1 \times (\text{albumin [g/L]}) + 8 \times \text{ascites (0 = no ascites; 1 = presence of ascites)} - 55]/10$ .

the nontreated group. All patients had complete biochemical data regarding baseline and 1-year serum bilirubin, ALP and AST serum values, whereas serum albumin concentrations at 1 year were available in 170 (79.1%) patients (UDCA: 79; 41.6%, non-UDCA: 91; 53.5%). After 1 year of UDCA, ALP and AST decreased significantly (*P* = 0.0001 for both; Table 3a), whereas all but one patient (which decreased albumin to 34 g/L) maintained normal bilirubin and albumin concentrations. In untreated patients, median bilirubin

concentration increased (*P* = 0.001), whereas ALP, AST and albumin remained largely unchanged (*P* = 0.15, 0.90 and 0.15 respectively; Table 3a). Overall, 14/129 (10.8%) untreated patients did not maintain nonhistological definition of early disease at 1 year: bilirubin increased above ULN in 13 patients, and 1 patient showed increased bilirubin and reduced albumin; all 13 had either stage III (4) or stage IV (5), except 4 without biopsy. When excluding patients with known Scheuer's III/IV stage (as not balanced between untreated and UDCA groups), there was also a reduction in ALP concentration in untreated patients (*P* = 0.05, Table 3b). Adverse outcomes occurred in 34 (15.8%); 26 untreated/8 UDCA (*P* = 0.04): liver-related death 9 (4.2%); 8 untreated/1 UDCA (*P* = 0.09); complications 18 (8.4%): 12 untreated (ascites 6, variceal bleeding 4, encephalopathy 1, HCC 1)/6 UDCA (ascites 4, variceal bleeding 2) (*P* = 0.62); and liver transplantation 7 (3.3%): 5 untreated/2 UDCA (*P* = 0.70). The survival rates without adverse outcome at 5, 10, 15 and 20 years were 92.2%/80.1%/74.9%/59.3%, respectively, for untreated patients, vs. 93.6% at 5 and 10 years and 87.4% at 15 and 20 years for UDCA-treated group (*P* = 0.11). Excluding late histological stages, an adverse outcome was documented for 20/185 (10.8%): 15 untreated/5 UDCA (*P* = 0.15), comprising a first complication in 14:10 untreated/4 UDCA (*P* = 0.40), liver transplantation in 4:3 untreated/1 UDCA (*P* = 0.64) and liver-related death in 2 (both untreated, *P* = 0.51). Survival rates free of an adverse event at 5, 10, 15 and 20 years were 95.8%/84.9%/78% and 56.7%, respectively, if untreated, vs. 98.7% at 5 years, and 93.8% at 10, 15 and 20 years with UDCA (*P* = 0.03).

### One-year biochemical features according to BA, PA-I, TO and PA-II definitions and correlated factors

Comparative attainments of 1-year biochemical definitions, between untreated and UDCA patients, are shown in Figure 1. Biochemical response to UDCA ranged from 51.2% to 76.7%, whereas 36.4–66.7% of untreated patients spontaneously fulfilled response definitions. Significant differences between UDCA and untreated groups were found for attaining BA (*P* = 0.03) and TO (*P* = 0.04) criteria (Figure 1a). However, there were no statistically significant differences between the two groups when excluding patients with known late baseline histology (Figure 1b), or considering those with less prominent baseline biochemical abnormalities: ALP ≤3x ULN (Figure 1c) or AST ≤2x ULN (Figure 1d).



**Table 3** | Biochemical characteristics in patients with early PBC (normal baseline bilirubin and albumin), at presentation and after 1 year from study entry: (a) total cohort ( $n = 215$ ), and (b) after exclusion of patients with late histological stages (Scheuer's III-IV) on baseline liver biopsy ( $n = 185$ ). Values are given as median (range)

	Reference range	At presentation	After 1 year	<i>P</i> -value*	At presentation	After 1 year	<i>P</i> -value*
		Untreated ( $n = 129$ )			UDCA-treated ( $n = 86$ )		
a)							
Total bilirubin ( $\mu\text{mol/L}$ )	5–17	9 (3–16)	9 (2–133)	0.001	10 (5–17)	10 (5–21)	0.35
ALP (U/L)	42–128	240 (50–1730)	234 (59–990)	0.15	226.5 (42–1074)	180.5 (42–1220)	0.0001
AST (U/L)	5–40	44.5 (18–312)	45 (12–210)	0.90	58 (14–270)	45 (12–253)	0.0001
Albumin† (g/L)	35–50	42 (35–51)	42 (22–51)	0.15	42 (35–57)	42 (34–57)	0.78
		Untreated ( $n = 107$ )			UDCA-treated ( $n = 78$ )		
b)							
Total bilirubin ( $\mu\text{mol/L}$ )		8 (3–16)	9 (2–133)	0.009	9 (5–17)	10 (5–21)	0.22
ALP (U/L)		214 (50–1730)	210 (59–990)	0.05	225 (42–1074)	178 (42–1220)	0.0001
AST (U/L)		43 (18–201)	45 (12–210)	0.51	58 (18–180)	45 (12–177)	0.0001
Albumin‡ (g/L)		43 (35–51)	42 (22–51)	0.15	42 (35–57)	42 (34–57)	0.70

PBC, primary biliary cirrhosis; UDCA, ursodeoxycholic acid; ALP, alkaline phosphatase; AST, aspartate aminotransferase.

\* *P*-values are based on Wilcoxon signed-rank test for paired data.

† Serum albumin concentrations at 1 year available in 170/215 (79.1%) patients.

‡ Serum albumin concentrations at 1 year available in 167/185 (90.3%) patients.

**Univariate and multivariate analysis.** Univariate and multivariate associations for the attainment of each biochemical response definition are shown in Table 4. Depending on the definition, baseline ALP  $\leq 3x$  ULN, AST  $\leq 2x$  ULN and early histology (Scheuer's I/II) remained independently associated with attainment of biochemical criteria. Receiving UDCA was independently associated with attaining BA ( $P = 0.03$ ), TO ( $P = 0.01$ ) and PA-II ( $P = 0.02$ ), but not PA-I criteria ( $P = 0.24$ ). Excluding baseline Scheuer's III–IV stages, both PA-I ( $P = 0.29$ ) and TO response criteria ( $P = 0.08$ ) occurred irrespective of receiving UDCA (Table 4b). Dosage of UDCA was not significantly associated with attaining biochemical criteria, even after excluding late-stage cases (Table 4a,b).

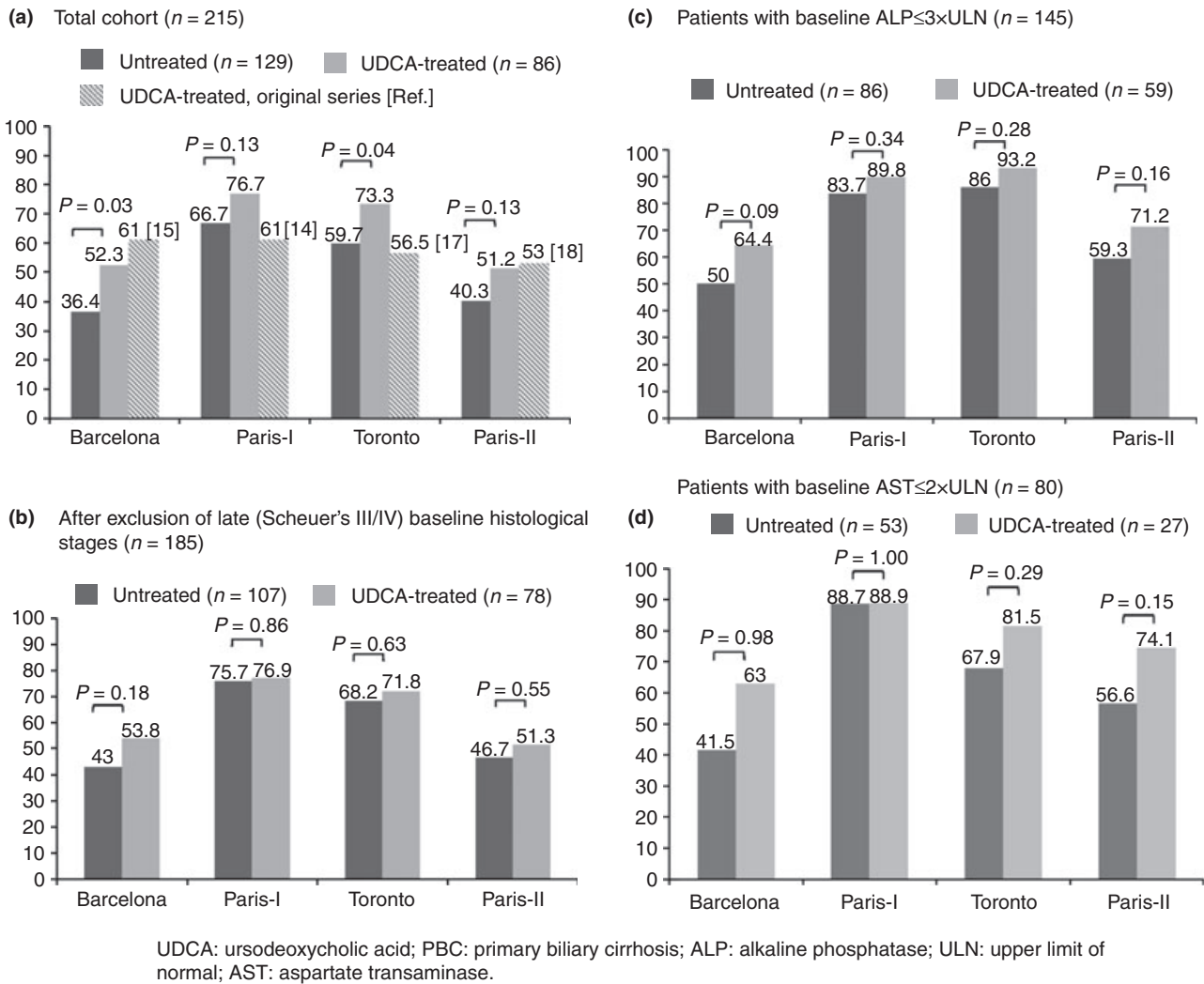
#### BA, PA-I, TO and PA-II as predictors of long-term outcomes in early PBC

Curves for survival without an adverse outcome showed a nonstatistically significant trend for better 5-, 10- and 15-year outcomes in responders vs. nonresponders to UDCA (Figure 2a); BA: 94.9% at all time-points vs. 92.3%/82%/82%; ( $P = 0.21$ ), PA-I: 100%/92.4%/92.4% vs. 100%/100%/70%; ( $P = 0.42$ ), TO: 95.7%/89.3%/89.3% vs. 92.6%/82.6%/82.6%; ( $P = 0.74$ ) and PA-II: 100%/96%/96% vs. 100%/92.7%/79.6% respectively ( $P = 0.28$ ). Excluding late baseline histology gave similar results:  $P = 0.09$  for BA, 0.74 for PA-I, 0.11 for PA-II and 0.20

for TO criteria (data not shown). In untreated patients, failure to attain PA-I criteria at 1 year was significantly associated with poor outcome (HR: 2.83, 95% CI: 1.24–6.45,  $P = 0.01$ ) (Figure 2b), whereas no criterion was predictive after excluding late-staged cases. Cox regression analysis in the evaluation of an adverse outcome included all variables in Table 4a and in addition: pruritus at baseline, AMA positivity and variables at 1 year (bilirubin  $>$  ULN, absence of BA/PA-I/PA-II/TO criteria). Age at presentation (HR: 0.93, 95% CI: 0.89–0.96;  $P = 0.0001$ ), 1-year bilirubin  $>$  ULN (HR: 5.64, 95% CI: 1.40–22.66;  $P = 0.01$ ) and late histological stage (HR: 5.18, 95% CI: 2.19–12.25;  $P = 0.0001$ ) were independent associations. Excluding late histological stages, 1-year bilirubin  $>$  ULN (HR: 3.94, 95% CI: 1.09–14.13;  $P = 0.03$ ) remained as the only independent predictor of poor outcome.

## DISCUSSION

Our study is the first to include an appropriate untreated cohort, specifically concerned with evaluating 1-year biochemical definitions as therapeutic surrogates in early PBC. We found that 1-year biochemical features described in UDCA response criteria occur spontaneously in 36.4%–66.7% of early PBC patients; rates which are not far different from those with UDCA in our own and published series (Figure 1a). When excluding patients with



**Figure 1** | Rates (%) of attainment of 1-year biochemical features fulfilling UDCA response definitions among 215 patients with early PBC (normal baseline bilirubin and albumin) untreated or UDCA-treated: (a) whole cohort (n = 215); UDCA response rates in the original Barcelona, Paris-I, Toronto and Paris-II series are shown for comparison, (b) excluding late histological stages (n = 185), (c) patients with baseline ALP ≤ 3x ULN (n = 145), (d) patients with baseline AST ≤ 2x ULN (n = 80).

late histology (known to adversely affect UDCA response,<sup>26</sup> and due to its imbalance between untreated and UDCA groups), or considering those with less abnormal baseline ALP or AST values, the attainment rates for all four biochemical definitions were statistically comparable between untreated and UDCA-treated patients (Figure 1b,c,d). Inconsistent performance of UDCA response criteria in early PBC was previously shown in the Dutch PBC study,<sup>16</sup> where survival of nonresponders was comparable to responders when the PA-I ( $P = 0.30$ ), BA ( $P = 0.96$ ) and Rotterdam criteria ( $P = 0.43$ ) were applied to 225 histologically defined early PBC patients. Similarly, a recent evaluation of the reliability of BA, PA-I, TO and

Rotterdam definitions in early disease showed poor positive (0.3–1.7) and negative (0.8–3.6) likelihood ratios, and therefore poor discrimination for either high- or low-risk patients.<sup>18</sup> In line with these older observations, and using complication- and transplantation-free liver-related survival as an endpoint, we could not identify any significant associations (Figure 2a). However, our relatively small sample of UDCA-treated patients ( $n = 86$ ) may lack sufficient statistical power to detect significance, as indicated by a trend for better long-term outcomes in UDCA responders. Importantly, even traditional prognostic models such as the Mayo<sup>27</sup> or Royal Free models<sup>20</sup> are not useful for early PBC, as most patients die from

**Table 4 |** Univariate and multivariate associations for attainment of 1-year biochemical features, as described in Barcelona, Paris-I, Toronto and Paris-II definitions: (a) in a cohort of 215 PBC patients with normal baseline bilirubin and albumin concentrations, and (b) after exclusion of patients with late (Scheuer's III and IV) baseline histology ( $n = 185$ )

Variable	Univariate <i>P</i> -value	Multivariate <i>P</i> -value	OR (95% CI)
(a)			
Barcelona			
Age	0.64	–	–
Gender	1.00	–	–
UDCA treatment	0.03	0.03	2.16 (1.07–4.36)
Baseline ALP $\leq 3 \times$ ULN	0.0001	0.0001	4.80 (2.18–10.58)
Baseline AST $\leq 2 \times$ ULN	0.20	–	–
Baseline histological stage: Scheuer's I and II	0.001	0.02	3.87 (1.19–12.6)
High/Standard UDCA dose*	0.38	–	–
Paris-I			
Age	0.28	–	–
Gender	0.17	–	–
UDCA treatment	0.13	0.24	1.62 (0.72–3.63)
Baseline ALP $\leq 3 \times$ ULN	0.0001	0.0001	9.95 (4.13–23.98)
Baseline AST $\leq 2 \times$ ULN	0.0001	0.0001	9.34 (3.10–28.09)
Baseline histological stage: Scheuer's I and II	0.0001	0.015	3.67 (1.23–9.1)
High/Standard UDCA dose*	0.43	–	–
Toronto			
Age	0.48	–	–
Gender	0.50	–	–
UDCA treatment	0.04	0.01	3.59 (1.35–9.53)
Baseline ALP $\leq 3 \times$ ULN	0.0001	0.0001	35.9 (13.71–94.30)
Baseline AST $\leq 2 \times$ ULN	0.10	–	–
Baseline histological stage: Scheuer's I and II	0.004	0.42	1.49 (0.42–5.29)
High/Standard UDCA dose*	0.33	–	–
Paris-II			
Age	0.51	–	–
Gender	0.52	–	–
UDCA treatment	0.13	0.02	2.84 (1.17–6.90)
Baseline ALP $\leq 3 \times$ ULN	0.0001	0.0001	34.23 (9.20–127.34)
Baseline AST $\leq 2 \times$ ULN	0.0001	0.0001	5.63 (2.23–14.22)
Baseline histological stage: Scheuer's I and II	0.013	0.71	1.27 (0.36–4.49)
High/Standard UDCA dose*	0.23	–	–
(b)			
Barcelona			
Age	0.63	–	–
Gender	1.00	–	–
UDCA treatment	0.18	0.05	1.92 (1.01–3.64)
Baseline ALP $\leq 3 \times$ ULN	0.0001	0.0001	6.33 (2.86–13.98)
Baseline AST $\leq 2 \times$ ULN	0.23	–	–
High/Standard UDCA dose†	0.64	–	–
Paris-I			
Age	0.26	–	–
Gender	0.22	–	–
UDCA treatment	0.86	0.29	1.55 (0.69–3.49)
Baseline ALP $\leq 3 \times$ ULN	0.0001	0.0001	7.97 (3.57–17.80)
Baseline AST $\leq 2 \times$ ULN	0.0001	0.0001	5.71 (2.01–16.26)
High/Standard UDCA dose†	0.10	–	–
Toronto			
Age	0.44	–	–
Gender	0.75	–	–

Table 4 | (Continued)

Variable	Univariate P-value	Multivariate P-value	OR (95% CI)
UDCA treatment	0.63	0.08	2.34 (0.91–6.03)
Baseline ALP $\leq$ 3 $\times$ ULN	0.0001	0.0001	38.01 (14.52–99.49)
Baseline AST $\leq$ 2 $\times$ ULN	0.03	0.67	1.22 (0.49–3.03)
High/Standard UDCA dose†	0.20	–	–
Paris-II			
Age	0.49	–	–
Gender	0.50	–	–
UDCA treatment	0.55	0.04	2.19 (1.03–4.70)
Baseline ALP $\leq$ 3 $\times$ ULN	0.0001	0.0001	31.89 (8.91–114.18)
Baseline AST $\leq$ 2 $\times$ ULN	0.0001	0.0001	3.92 (1.83–8.41)
High/Standard UDCA dose†	0.10	–	–

PBC, primary biliary cirrhosis; UDCA, ursodeoxycholic acid; ALP, alkaline phosphatase; AST, aspartate transaminase; ULN, upper normal limit; OR, odds ratio; CI, confidence interval.

\* 13–15 mg/kg/day; analysis regards 86 patients with UDCA treatment.

† 13–15 mg/kg/day; analysis regards 78 patients with UDCA treatment.

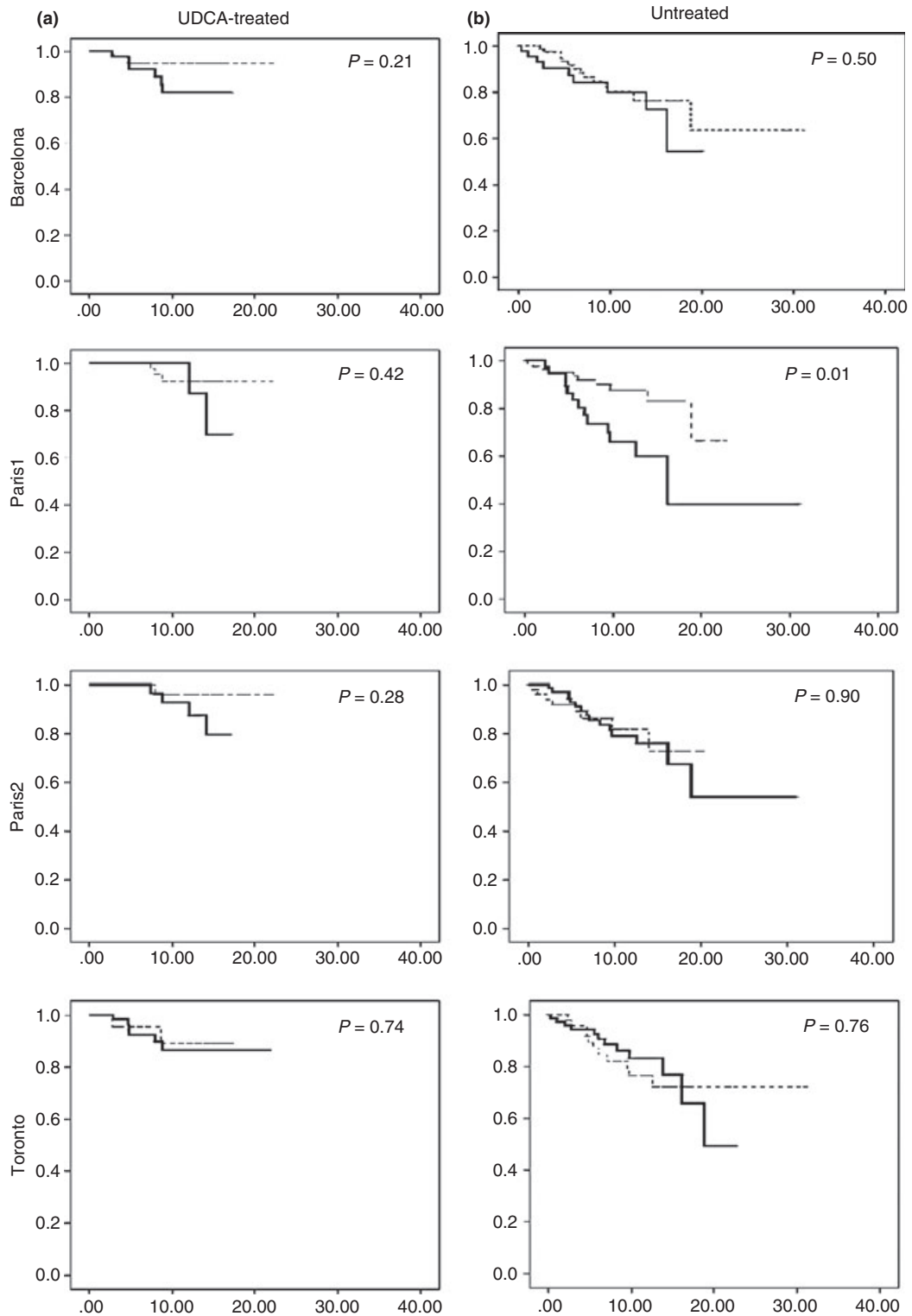
nonliver-related causes<sup>28</sup> and only a minority have liver-related deaths or LT. In a previous study,<sup>16</sup> baseline bilirubin and albumin concentrations, both classic biochemical predictors in PBC, were prognostically irrelevant in early disease, highlighting difficulties for accurate prognostication in this disease category. To our results, baseline late histology, 1-year bilirubin  $>$ ULN and inversely increasing age were the only independent predictors of a poor outcome. A recent study also found an inverse association with age with respect to UDCA response and symptoms.<sup>27</sup>

PA-I are considered the best validated criteria, recommended in clinical trials.<sup>29</sup> In our early PBC cohort, attainment of PA-I criteria was independent of UDCA treatment, suggesting that the association between PA-I criteria and prognosis is not driven by UDCA. Notably, when patients with late histology were excluded, fulfilment of TO criteria also occurred irrespective of UDCA (however, there was a 1-year evaluation in our study vs. evaluation for 2 years originally<sup>17</sup>). Congruent with our findings that causal association of biochemical response is suboptimal for UDCA, PA-I criteria were predictive of long-term outcome in untreated patients with early PBC (Figure 2b), confirming data in abstract form.<sup>19</sup>

More recently, the PA-II criteria have been proposed as the most reliable in early PBC,<sup>18</sup> derived from 165 patients with early histology, using liver-related survival or any clinical or histological evidence of development of cirrhosis as endpoints.<sup>18</sup> These criteria were also valid for a nonhistological definition of early disease.<sup>18</sup> We evaluated both histological and nonhistological criteria

and found comparable results. Our patients with PA-II response had a survival at 5, 10 and 15 years without an adverse outcome of 100%, 96% and 96%, respectively, compared to 95% at all time-points originally published,<sup>18</sup> if early disease was defined histologically. With the nonhistological definition of early PBC, both the original cohort<sup>18</sup> and our own had a 100% survival without an adverse outcome. The same number of liver-related deaths, LT or development of complications ( $n = 8$ ) occurred in both studies. However, although PA-II criteria were specifically developed in early PBC, our data clearly question the response criteria as adequately reflecting the therapeutic benefit of UDCA. This was shown in multivariate analysis in which biochemical features compatible with 'treatment response' were largely related to baseline factors reflecting severity of the disease (ALP/AST levels and histological stage) and to a far lesser extent, or not at all for some criteria, to UDCA (Table 4). Notably, baseline ALP and AST were categorised using thresholds of  $\leq 3x$  ULN and  $\leq 2x$  ULN, respectively, as used in PA-I criteria<sup>14</sup> (incorporating higher ALP/AST thresholds), corresponding to patients with baseline biochemistry likely to fulfil at least one response criteria. Thus, if at baseline the thresholds for response criteria are already present, then this confers a likelihood to maintain them at 1 year irrespective of therapy. Notably, 37.7% of our cohort had a baseline ALP  $\leq 1.5x$  ULN. This subpopulation of early PBC patients diagnosed histologically (poorly represented in previous studies evaluating UDCA responses<sup>14, 15, 17, 18</sup>) has influenced our results, as most maintain the criteria for 1 year irrespec-





**Figure 2** | Kaplan–Meier plots of survival without adverse outcome (liver-related death, LT, complications) in patients with early PBC (normal baseline bilirubin and albumin), according to 1-year biochemical criteria (Barcelona, Paris-I, Paris-II and Toronto) attained: (a) after 1 year of UDCA therapy ( $n = 86$ ), and (b) by natural biochemical variation in patients with no treatment ( $n = 129$ ). Dotted curves represent patients attaining criteria; solid curves represent patients not attaining the criteria. LT, liver transplantation; PBC, primary biliary cirrhosis; UDCA, ursodeoxycholic acid.

tive of receiving any treatment. However, spontaneous biochemical improvement also occurred, fulfilling UDCA response criteria in untreated patients with more pronounced baseline biochemical abnormalities (ALP  $\leq 3$ x ULN or AST  $\leq 2$ x ULN) in whom attainment rates for some criteria exceeded 80% (Figure 1c,d). Critically, our data suggest that the therapeutic benefit with UDCA may have been overestimated in early PBC. This may provide an explanation as to why almost 2/3 of PBC patients achieve responses with UDCA, but only approximately 25% achieve 15-year survival without an adverse outcome, including histological progression to late stages.<sup>14, 18</sup> Given the slowly evolving character of the disease, longer evaluation periods (2–3 years, or more) may be necessary for a thorough assessment of UDCA response in early PBC. However, such subanalysis was not feasible with our data, as serial biochemical testing at time-points later than 1 year from UDCA start was not available. With current criteria, the optimal timing for UDCA response assessment in early PBC remains to be addressed in future studies.

Our study has limitations mainly concerning ‘suboptimal’ UDCA dosing in some patients, although we could not detect any statistically significant effect in either biochemical or clinical endpoints. Secondly, we observed only a 20% reduction in median ALP concentration, whereas it was 50% in the PA-II study.<sup>18</sup> However, our median baseline ALP levels were 226.5 U/L (only 1.8x ULN) vs. a mean of 3.6x ULN for PA-II cohort,<sup>18</sup> precluding a more pronounced UDCA-induced reduction in ALP serum concentrations in our population, because of lower baseline values. Despite this, our overall UDCA response rates are not markedly different from those in the original cohorts, i.e. 61% for both BA and PA-I criteria,<sup>14, 15</sup> 56.5% for TO<sup>17</sup> and 53% for PA-II<sup>18</sup> (Figure 1a). The higher rate of PA-I response (76.7% vs. 61% originally<sup>14</sup>) is probably accounted by fewer patients with late histology (18.5% vs. 45%). Contrarily, our late histology rate is comparable to that in TO (21.7%) and BA (23.7%)

series, even though neither study was specifically concerned with early disease.

In conclusion, our data show that current criteria for assessing response to UDCA are not robust enough for use in early PBC. Biochemical responses at 1 year give a good measure of disease severity, and thus may represent a reliable predictor of long-term prognosis, but provide little or no information on response to UDCA. Our findings demonstrate the need to further refine biochemical response criteria to establish a reliable causal link between outcomes and treatment effects. This will allow accurate patient selection for future clinical trials, helping efforts for developing new therapeutic approaches in PBC.

### AUTHORSHIP

*Guarantor of the article:* Andrew K. Burroughs.

*Author contributions:* VP, MRP, ET, GP, PM, GG, CR, VA collected data. VP, EAT and AKB designed the study and review each draft of the publication. VP performed statistical analyses and wrote the manuscript. EAT, MRP, SK and AKB contributed to interpretation and analyses of data. EAT, SK and AKB critically revised the article for important intellectual content. All authors approved the final version of the manuscript.

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### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Baseline characteristics of consecutive patients with early primary biliary cirrhosis (normal baseline bilirubin and albumin): (a) considering the whole cohort and (b) after excluding patients ( $n = 30$ ) with late (Scheuer’s III and IV) baseline histology.

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