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Measurement of Gamma Glutamyl Transferase to Determine Risk of Liver Transplantation or Death in Patients With Primary Biliary Cholangitis

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(Article begins on next page)

Title: Measurement of Gamma Glutamyl Transferase to Determine Risk of Liver Transplantation or Death in Patients With Primary Biliary Cholangitis

Short Title: The prognostic role of GGT in PBC

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List of Abbreviations:

Alkaline phosphatase (ALP)

Alanine aminotransferase (ALT)

Antimitochondrial antibodies (AMA)

Aspartate aminotransferase (AST)

Area Under the Receiver Operating Characteristic (AUROC)

European Association for the Study of the Liver (EASL)

Gamma-glutamyl transferase (GGT)

Hazard ratio (HR)

Liver transplantation (LT)

Lower limit of normal (LLN)

Primary Biliary Cholangitis (PBC)

Receiver Operating Characteristics (ROC)

Ursodeoxycholic Acid (UDCA)

Upper limit of normal (ULN)

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Abstract

Background and Aims: Gamma-glutamyltransferase (GGT) is a serum marker of cholestasis. We investigated whether serum level of GGT is a prognostic marker for patients with primary biliary cholangitis (PBC).

Methods:

We analyzed data from patients with PBC from the Global PBC Study Group, comprising 14 centers in Europe and North America. We obtained measurements of serum GGT at baseline and time points after treatment. We used Cox model hazard ratios to evaluate the association between GGT and clinical outcomes, including liver transplantation and liver-related death.

Results:

Of the 2129 patients included in our analysis, 281 (13%) had a liver-related clinical endpoint. Mean age at diagnosis was 53 years and 91% of patients were female patients. We found a correlation between serum levels of GGT and alkaline phosphatase (ALP) ($r=0.71$). Based on data collected at baseline and yearly for up to 5 years, higher serum level of GGT were associated with lower hazard for transplant-free survival. Serum level of GGT at 12 months after treatment higher than 3.2-fold the upper limit of normal (ULN) identified patients who required liver transplantation or with liver-related death at 10 years with an area under the receiver operating characteristic curve of 0.70. The risk of liver transplantation or liver-related death in patients with serum level of GGT above 3.2-fold the ULN, despite level of ALP lower than 1.5-fold the ULN, was higher compared to patients with level of GGT lower than 3.2-fold the ULN and level of ALP lower than 1.5-fold the ULN ($P<.05$). Including information on level of GGT increased the prognostic value of the Globe score.

Conclusion: Serum level of GGT can be used to identify patients with PBC at risk for liver transplantation or death, and increase the prognostic value of ALP measurement. Our findings support the use of GGT as primary clinical endpoint in clinical trials. In patients with low serum

level of ALP, a high level of GGT identifies those who might require treatment of metabolic disorders or treatment escalation.

Keywords: UDCA; biomarkers; autoimmune liver disease; prediction.

Journal Pre-proof

Introduction

Primary biliary cholangitis (PBC) is a chronic disease characterised by an autoimmune damage of the small bile ducts. The disease course is typically slow and progressive, and can evolve to cirrhosis and its complications if untreated or undertreated. The backbone of treatment is ursodeoxycholic acid (UDCA); however, up to 40% of all UDCA-treated subjects have inadequate response by current definitions¹. Lack of biochemical response in PBC is strongly associated with reduced survival, and identification of patients at higher-risk of poor outcomes is essential to identify those who can benefit from treatment escalation²⁻⁵. Different risk stratification tools already exist, and include clinical variables^{4,6,7}. In all existing models, markers are integrated to estimate the risk of death or liver transplantation (LT) typically after 12 months of optimally-dosed treatment with UDCA. Total bilirubin and alkaline phosphatase (ALP) are the main biochemical parameters used for diagnostic and prognostic purposes in PBC, and are present in all validated scores⁶⁻⁸. Yet, another serum marker, gamma-glutamyl transferase (GGT), is also typically elevated in chronic cholestasis. While European Association for the Study of the Liver (EASL) clinical practice guidelines suggest to use GGT to confirm PBC diagnosis¹, its role in defining prognosis and predicting treatment response has never been proven.

GGT is a liver-specific enzyme associated with cholestasis. It can also increase secondary to drug or alcohol exposure or fatty liver in the presence of metabolic comorbidities (e.g. diabetes, obesity)⁹. In contrast, ALP is not a liver-specific marker¹⁰ and can be raised in osteoporosis, condition frequently associated with PBC¹¹, in pregnancy and childhood. Isolated raised ALP levels with normal GGT levels are rarely found in cholestasis.

In previous landmark clinical trials evaluating the efficacy of second-line drugs in PBC, ALP was used as treatment endpoint; nevertheless, the behaviour of GGT in treated patients mirrored that of ALP^{12,13}. There is evidence that farnesoid X agonists induce bone-derived ALP gene

expression^{14,15}, possibly making ALP values less reliable. Interestingly, there are a few novel drugs currently investigated in clinical trials including patients with PBC which have used reduction of GGT levels, instead of ALP levels, as primary outcome¹⁶⁻¹⁸. Nonetheless, it is not clear whether the achievement of a reduction in GGT levels translates into meaningful positive long-term clinical outcomes. Indeed, little is known about the prognostic role of GGT and its relation to ALP in patients with PBC.

The aim of our study was to explore whether levels of GGT at different time points can be an accurate predictor of liver-related outcomes in patients with PBC.

Patients and Methods

Study Design and Population

This is a multicenter, international, observational cohort study. We used data from the Global PBC Study Group multicenter cohort. The cohort has been described in detail elsewhere⁸. Briefly, PBC was defined according to established international guidelines¹. Demographic, clinical and outcome data were collected from 14 centers across Europe and North America.

We included in this study UDCA-treated and untreated patients present in the database with available GGT at 12 months of follow-up.

Patients were excluded from the analysis if they had a short follow-up (< 6 months), data on GGT after 12 months of treatment with UDCA were unavailable, or whether the start date of treatment or the exact date of major clinical events was unknown, or if they had concomitant liver disease (e.g. overlap with viral hepatitis or autoimmune hepatitis).

The primary outcome was a composite endpoint including either LT or death. Both all-cause death and liver-related death were evaluated in the composite outcome.

Explanatory variables

The following variables were available: age at diagnosis, sex, year of diagnosis, treatment with UDCA, histological stage (Scheuer and Ludwig stages), blood tests at the time of diagnosis, i.e. GGT, ALP, alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, albumin, antimitochondrial antibodies (AMA) status.

In order to account for the inter-laboratory variability GGT, ALP, ALT, AST, bilirubin values were normalized according to the higher reference values – and albumin to the lower reference values – and expressed as ratio (x upper limit of normal, ULN; x lower limit of normal, LLN).

Ethical Approval

This study was conducted in accordance with the protocol and the principles of the Declaration of Helsinki. The protocol was approved by each participating center in accordance with local regulations.

Statistical analysis

Study entry was defined as the start date of UDCA therapy, or the date of the first center visit for patients not treated with UDCA. Patients without documented events during follow-up were censored at their last follow-up visit.

We analysed the correlation between GGT and ALP using Spearman's correlation index at baseline and yearly up to five years of follow-up. We also analysed the correlation between 'relative delta (Δ) variations' of GGT and ALP before and after treatment - Δ GGT defined as: $(GGT \text{ levels at baseline} - GGT \text{ levels at 12 months})/GGT \text{ levels at baseline}$, and Δ ALP defined as: $(ALP \text{ levels at baseline} - ALP \text{ levels at 12})/ALP \text{ levels at baseline}$. All analyses evaluating relative Δ variations were performed on a sub-cohort of patients without missing GGT values at entry of the study and 12 months later and treated with UDCA.

To study the association between GGT and outcome, the Cox model hazard ratios (HRs) of LT or death were estimated by applying a cubic spline function of GGT at baseline and yearly up to 5

years of follow-up. To further evaluate the association of GGT with LT-free survival, the effect of GGT was adjusted for center, year of diagnosis (categorized in before 1990 and after 1990), age at diagnosis, UDCA therapy, sex, and different biochemical variables. We explored interactions between GGT and ALP.

We undertook a Multiple Imputation with Chained Equations approach (5 imputations) to handle missing values of factors used as adjusting variables in the multivariate analysis, using the *MICE* package in *R*.

To investigate the predictive performance of GGT at 12 months of follow-up towards the two composite end-points (all-cause death or LT and liver-related death or LT) at 5 and 10 years of follow-up and to find the optimal cutoff to dichotomize GGT we used the time-dependent Receiver Operating Characteristic (ROC) curve method¹⁹.

To investigate whether the dichotomized GGT was a meaningful surrogate endpoint, we repeated Cox analyses for multiple subgroups of patients. Subgroups were defined by UDCA therapy, histological disease stage (dichotomized in Ludwig or Scheuer 1 and 2 (early) or Ludwig and Scheuer 3 and 4 (advanced)), biochemical disease stage (according to Rotterdam criteria, dichotomized in early versus moderate and advanced), age at time of diagnosis (< or \geq 45 years), sex, and date of diagnosis (before 1990 or after 1990).

LT-free survival was assessed for the dichotomized GGT and for a combination of GGT and ALP by Kaplan–Meier estimates. Log-rank test was used for comparisons between groups. The combination of GGT and ALP was meant to explore whether the addition of GGT could improve risk stratification of PBC patients. ALP threshold used in this analysis was equal to 1.5 x ULN, in accordance to EASL CPG definition of low-risk patient¹.

Survival analyses, including time-dependent ROC curve analysis, Kaplan-Meier estimates and Log-rank test were repeated also for Δ GGT and Δ ALP.

Finally, to explore the average trend of GGT/total bilirubin and ALP/GGT ratios over time in patients who experienced LT or death, we fitted a linear mixed model with random intercept and

random slope. The follow-up of patients was aligned at the time of occurrence of the composite endpoint, fixing the origin at that time and reversing the time axis.

Normally distributed data are presented as mean and standard deviation and skewed distributed data as median and interquartile range. A two-sided $P < 0.05$ was considered statistically significant.

All analyses were performed using R (v.3.5.1, R Core Team).

Results

Among 4245 patients included in the Global PBC database, 2129 met the inclusion criteria for this study (**Supplementary Figure 1, Supplementary Table 1**). Features of the cohort are shown in **Table 1** and missing data in **Supplementary Table 2**. Demographic characteristics at diagnosis were consistent with those reported in the literature. Over the period of follow-up, 406 subjects experienced a clinical endpoint: 288 died and 116 underwent LT. Liver-related deaths were 165; 123 patients (42.7% of deaths) died due to non-liver related causes. In the whole cohort, 5-, 10- and 15-year LT-free survival rates were 91%, 80% and 70%, respectively (**Supplementary Figure 2**).

Association between GGT levels and ALP levels

Correlation between GGT and ALP was strong, as proved by Spearman index ($r=0.71$) (**Figure 1**), up to five years of follow-up (**Supplementary Figure 3**). In the sub-cohort of patients with GGT values at entry of the study and at 12 months of UDCA ($n = 1630$), median Δ GGT was 0.63 (63% median reduction in GGT levels from baseline), while median Δ ALP was 0.35 (35% median reduction in ALP levels from baseline); we found a moderate correlation ($r=0.65$) between Δ GGT and Δ ALP (**Supplementary Figure 4**).

Association between GGT and the risk of LT or death

The association between GGT levels (at baseline and yearly up to 5 years of follow-up) and the risk of LT or death was log-linear, and higher levels of GGT were associated with reduced LT-free survival (**Figure 2A and 2B** and **Supplementary Figure 5 (A-D)**).

We fitted a Cox model including the variables present in the Global PBC score, i.e. ALP, bilirubin, platelets and albumin levels evaluated at 12 months and age at diagnosis, with the addition of GGT at 12 months. HR of LogGGT was 2.55 (95% CI 1.58, 4.10) and HR of LogALP was 2.54 (95% CI 1.27, 5.05) (**Table 2**).

We performed a time-dependent ROC analysis to identify the optimal cut-off of GGT and ALP at 12 months follow-up discriminating between patients experiencing the event and those free from the event at 10 years. The optimal cut-off for liver-related death or LT was 3.2 times the ULN for GGT, and 2.0 times the ULN for ALP (**Supplementary Figure 6**). The area under the ROC curve (AUROC) value was 0.70 for GGT, and 0.72 for ALP. The 5-,10- and 15-year LT-free survival rates for patients with GGT levels < 3.2 times the ULN were respectively 94%, 86% and 78%; for patients with GGT levels ≥ 3.2 times the ULN the same rates were 85%, 69% and 58% (**Supplementary Figure 7**).

The optimal cut-off for all-cause death or LT was 3.5 times the ULN for GGT, and 2.0 times the ULN for ALP (**Supplementary Figure 8**). The area under the ROC curve (AUROC) value was 0.66 for GGT, and 0.70 for ALP.

The discriminative power of GGT at 12 months was also assessed evaluating the outcome (liver-related death or LT) at different time points, from 2 year- to 15 year- follow-up (**Supplementary Figure 9**).

Sub-group analysis

We further explored the ability of GGT to predict outcome in different patient subgroups. The threshold of 3.2 times the ULN after 12 months of follow-up was still predictive in the following subgroups: patients treated with UDCA, patients with histologically early and late disease, patients

with biochemically early disease, patients younger or older than 45 years of age at diagnosis, females or males, diagnosed before 1990 or after 1990. GGT levels did not show a significant association with the outcome in the subgroups with low numbers/ few events, i.e. untreated patients and patients with biochemically moderate/advanced disease (**Figure 3**).

GGT improves risk stratification based on ALP

To explore the contribution of GGT in the risk stratification based on ALP, two groups (ALP ≥ 1.5 x ULN, i.e. high-risk; and ALP < 1.5 x ULN, i.e. low risk) were defined. The comparison of these two groups is shown in **Supplementary Figure 10**.

The risk of death or LT in patients with GGT levels ≥ 3.2 times the ULN, compared with those with GGT levels < 3.2 times the ULN, was higher in both those subjects with ALP < 1.5 times the ULN and ALP ≥ 1.5 times the ULN. Notably, in the ALP < 1.5 x ULN group, the 5-,10-,15-year LT-free rates for patients with GGT levels < 3.2 times the ULN were 96%, 90% and 83%, respectively; in the same group, the rates for patients with GGT levels ≥ 3.2 times the ULN were significantly lower (90%, 83% and 74%, respectively; $p < 0.05$) (**Figure 4**).

Notably, findings were consistent and maintained when all-cause death or liver-related death were used (not shown), when analyses were restricted to only treated patients (**Supplementary Figure 11**) when using other ALP cutoffs (1.67 x ULN and 2.0 x ULN) in treated patients (**Supplementary Figure 12** and **13**) and when including only treated patients having ALP levels < 1.5 x ULN at 12 months and stratified by levels of bilirubin ($<$ or ≥ 1.0 x ULN) (**Supplementary Figure 14**).

Relative delta variations of GGT

To explore the possible predictive value of Δ GGT we repeated survival analyses in the selected cohort of UDCA treated patients with GGT values at both baseline and 12 months of follow-up (n =1630 subjects). In univariate Cox regression analysis, higher values of Δ GGT were associated with lower hazard of liver-related death or LT (HR 0.72, 0.62-0.83, $p < 0.001$); higher values of

Δ ALP did not associate with lower hazard of liver-related death or LT (HR 0.82, 0.65-1.04, $p = 0.10$). The optimal cut-off of Δ GGT able to discriminate between those patients experiencing liver-related death or LT, evaluated at 10 years, was 0.66 (66%); the AUROC value for Δ GGT was 0.68. The optimal cut-off of Δ ALP able to discriminate between those patients experiencing liver-related death or LT, evaluated at 10 years, was 0.45 (45%): the AUROC value for Δ ALP was 0.58 (**Supplementary Figure 15**).

Transplant-free survival was significantly worse in patients with Δ GGT values from baseline $< 66\%$ and in patients with Δ ALP from baseline $< 45\%$ (**Supplementary Figure 16 A and B**).

Trend of GGT/bilirubin and ALP/GGT ratios over disease course

We explored the behavioral pattern of two ratios, GGT/bilirubin and ALP/GGT, over time to investigate whether the relationship between GGT and the other two established prognostic markers changes as disease progresses. Averaging all subjects' trends, we found a progressive reduction of GGT/bilirubin ratio over time, reaching a value of 4.25 one year, 3.97 six months and 3.75 one month before the occurrence of LT or death, respectively (**Supplementary Figure 17A**). ALP/GGT ratio showed a slight increase from enrollment to time of the event, reaching a value of 0.86 one year before, 0.88 six months and 0.89 one month before the occurrence of LT or death (**Supplementary Figure 17B**).

Discussion

In this international, multicenter study, we provide evidence of the prognostic role of GGT in patients with PBC. We have shown that higher GGT values do associate with worse prognosis, and therefore GGT represents a clinical marker of long-term outcome in PBC. The prognostic power of GGT towards hard clinical endpoints (LT-free survival) is maintained irrespective of stage of the disease, sex and age. GGT levels identify a higher risk group among those currently considered at low risk based on ALP definition (< 1.5 times the ULN). Finally, GGT decreases over time as the disease progresses, as shown by the decrease of GGT/bilirubin ratio, which is due to reduction of GGT and increase of bilirubin at the same time, and the reduction of ALP/GGT ratio before LT or death; therefore the prognostic cut-off presented in this work should be applied only for bilirubin values under $1.0 \times$ ULN.

Abnormal GGT levels ($\geq 3.2 \times$ ULN) at 12 months discriminate the prognosis of patients with ALP $< 1.5 \times$ ULN, currently defined as low risk group by EASL recommendations¹. Interestingly, these patients (n=160) die of liver-related events rather than cardiovascular or other events (rate of non-liver-related deaths = 35% within this sub-cohort, which is comparable to 41% in the same rate within the group ALP $> 1.5 \times$ ULN and GGT $< 3.2 \times$ ULN, and far lower than 47% in patients with ALP $< 1.5 \times$ ULN and GGT $< 3.2 \times$ ULN). Moreover, we noticed that patients with elevated GGT and low ALP have higher inflammatory markers (ALT, AST) and ALP values than patients with low GGT and low ALP (**Supplementary Table 3**). We are not able to discriminate whether

patients with $GGT \geq 3.2 \times ULN$ and $ALP < 1.5 \times ULN$ suffer from overlap of PBC and fatty liver disease or, in alternative, GGT is a complimentary marker of cholestasis; in the latter case it might be necessary to identify lower thresholds for ALP and GGT, as proposed by Murillo et al²⁰. Future studies should better define the features of this subgroup of patients. In the meantime, PBC patients with low ALP and raised GGT levels should be thoroughly screened for common causes of isolated GGT elevation such as liver steatosis, alcohol abuse and medications; after this careful evaluation, escalation with anticholeretic drugs might be justified.

GGT is an orphan marker of cholestasis with unknown prognostic role in PBC so far. There is evidence supporting prognostic role of GGT in other cholestatic diseases in the pediatric setting²¹⁻²⁵, where GGT has been commonly used instead of ALP. Indeed, due to the changing values of the bone isoenzyme of ALP in growing children, clinical interpretation of laboratory values may be misled. Although PBC affects nearly only adults, it can be associated to conditions like osteoporosis where ALP levels may be non-specific²⁶. In postmenopausal women serum ALP levels are strongly correlated with bone ALP levels²⁶. This makes ALP in middle-aged women with PBC less specific. In the context of PBC and bone disease, GGT can be a valid marker of cholestasis to stratify risk of liver-related events, define disease activity and track response to therapy.

The choice of GGT as primary efficacy endpoint in Phase II clinical trials who evaluated the safety and efficacy of Setanaxib¹⁷ and Tropifexor¹⁶ in patients with PBC was driven by a context-specific biological rationale, despite its use as surrogate endpoint had not been validated. Our study offers a piece of evidence to support the use of GGT as reliable clinical endpoint for clinical trials in PBC.

Our results are not able to provide a clear threshold for clinical trials choosing GGT as primary efficacy outcome. However, the spline plots clearly show that the lower the GGT level, the better the outcome. Additionally, reduction of GGT levels under UDCA treatment equal or bigger than

66% from baseline is associated with better transplant-free survival; this could be of help when planning the percentage of decrease of GGT to be used as primary endpoint for a new drug.

Limitations of the study are its retrospective nature that prevented full data for all the variables. We were also not able to account for possible known confounders like alcohol and drug exposure or histological evidence of liver steatosis, since these data are not available in the Global PBC Study Group database. Yet, the strong correlation of GGT with ALP supports the concept that GGT is also a marker of cholestasis and offers complementary information to ALP.

In conclusion, our study shows that GGT is a solid prognostic marker of outcome in PBC, and that the higher the values of GGT the worse the LT-free survival. As 'Global PBC' investigators, we endorse the use of GGT together with validated risk scores to discriminate long-term prognosis of patients with PBC. Moreover, we believe that in patients with isolated elevation of GGT clinicians should at first screen and address common causes of GGT elevation such as metabolic liver disease and medications; if excluded, add-on therapies for PBC might be considered with the aim to reduce also GGT levels together with ALP and bilirubin levels. Finally, GGT can safely replace ALP in patients with conditions that may spuriously alter levels of ALP or in clinical trials when considered more appropriate by investigators.

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Tables

Table 1. Characteristics of the study population

		Total cohort
		(N = 2129)
Age at diagnosis		53.1± 12.1
Year of diagnosis	< 1990	510 (24.3)
	> 1990	1594 (75.7)
Female sex (%)		1932 (90.7)
AMA positive (%)		1888 (89.1)
Treated with UDCA (%)		1983 (94.2)
Histological disease stage^a (%)	1	533 (44.3)
	2	332 (27.6)
	3	221 (18.4)
	4	118 (9.8)
Biochemical disease stage^b (%)	early	1017 (72.3)
	moderately advanced	313 (22.3)
	advanced	76 (5.4)
Serum GGT at baseline (x ULN)		5.98 [3.12, 10.88]
Serum ALP at baseline (x ULN)		2.16 [1.34, 3.80]
Serum bilirubin at baseline (x ULN)		0.6 [0.45,1.00]
Serum AST at baseline (x ULN)		1.5 [1.00-2.24]
Serum ALT at baseline (x ULN)		1.6 [1.00-2.54]
Serum GGT at 12 months (x ULN)		2.09 [1.00, 4.60]
Serum ALP at 12 months (x ULN)		1.30 [0.89, 2.15]
Serum bilirubin at 12 months (x ULN)		0.58 [0.41, 0.83]
Serum albumin at 12 months (x LLN)		1.16 [1.08, 1.25]
Serum AST at 12 months (x ULN)		0.93 [0.70, 1.41]
Serum ALT at 12 months (x ULN)		0.89 [0.60, 1.50]
Median follow up (years)		7.50 [3.91, 12.18]
Liver transplants (%)		116 (5.4)
All-cause deaths (%)		288 (13.5)
Liver-related deaths (%)		165 (7.7)

^a histological disease stage according to Ludwig and Scheuer's classification

^b biochemical disease stage according to Rotterdam criteria

Abbreviations: AMA= anti mitochondrial antibodies; UDCA = ursodeoxycholic acid; GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

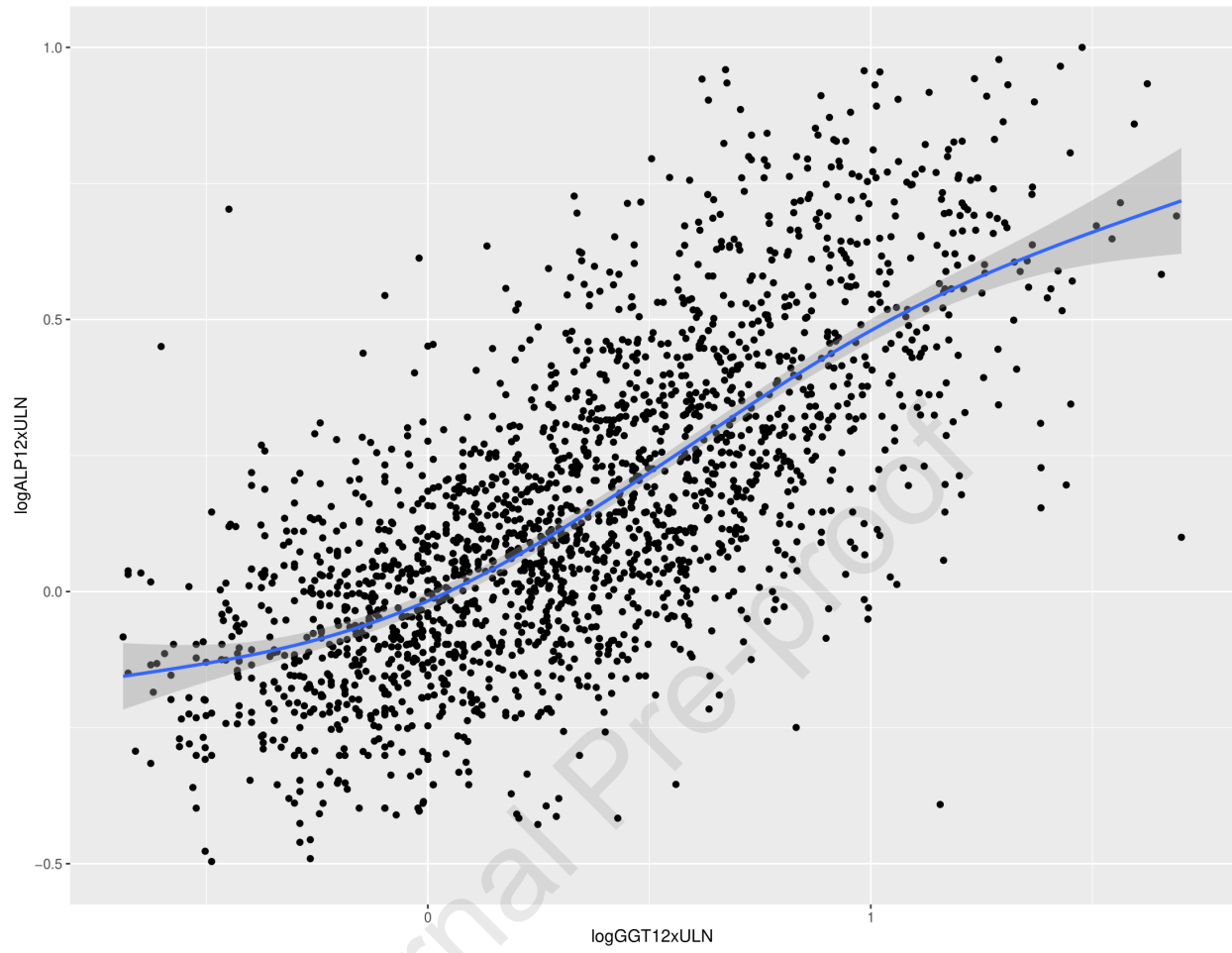
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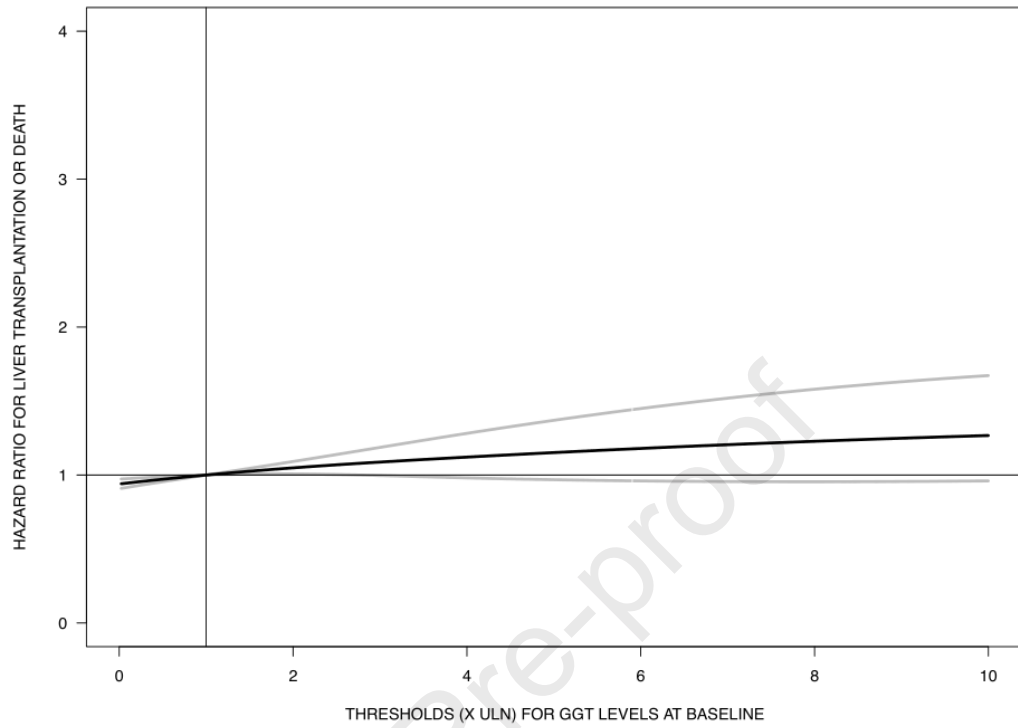
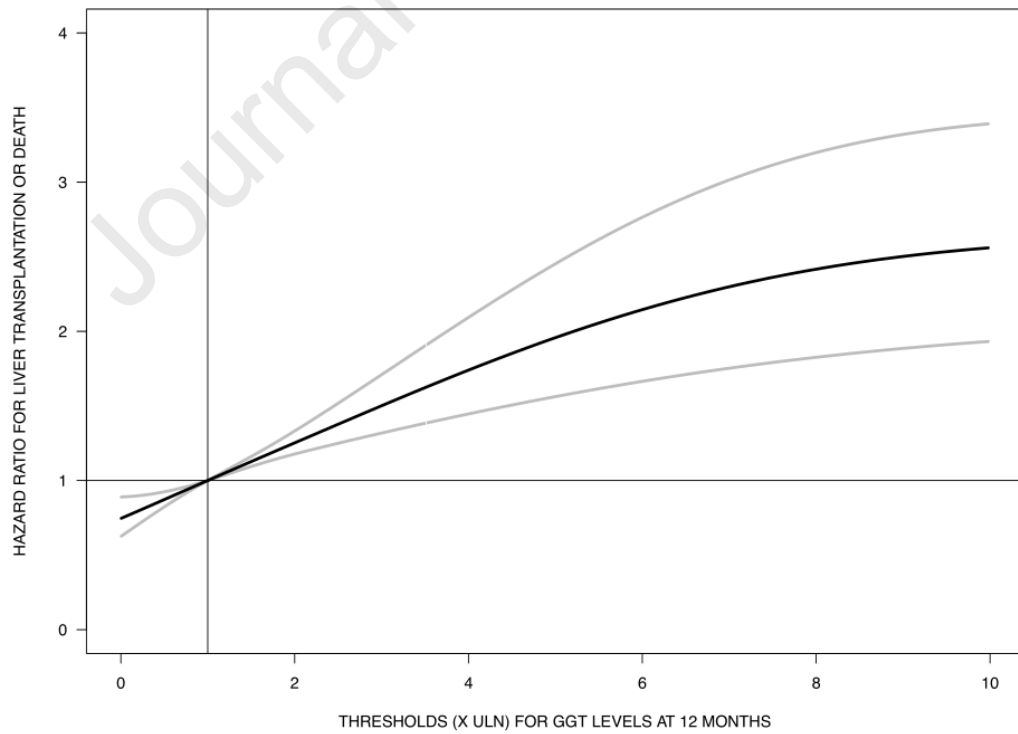
Table 2. Multivariate analysis to identify variables associated with LT or liver-related death

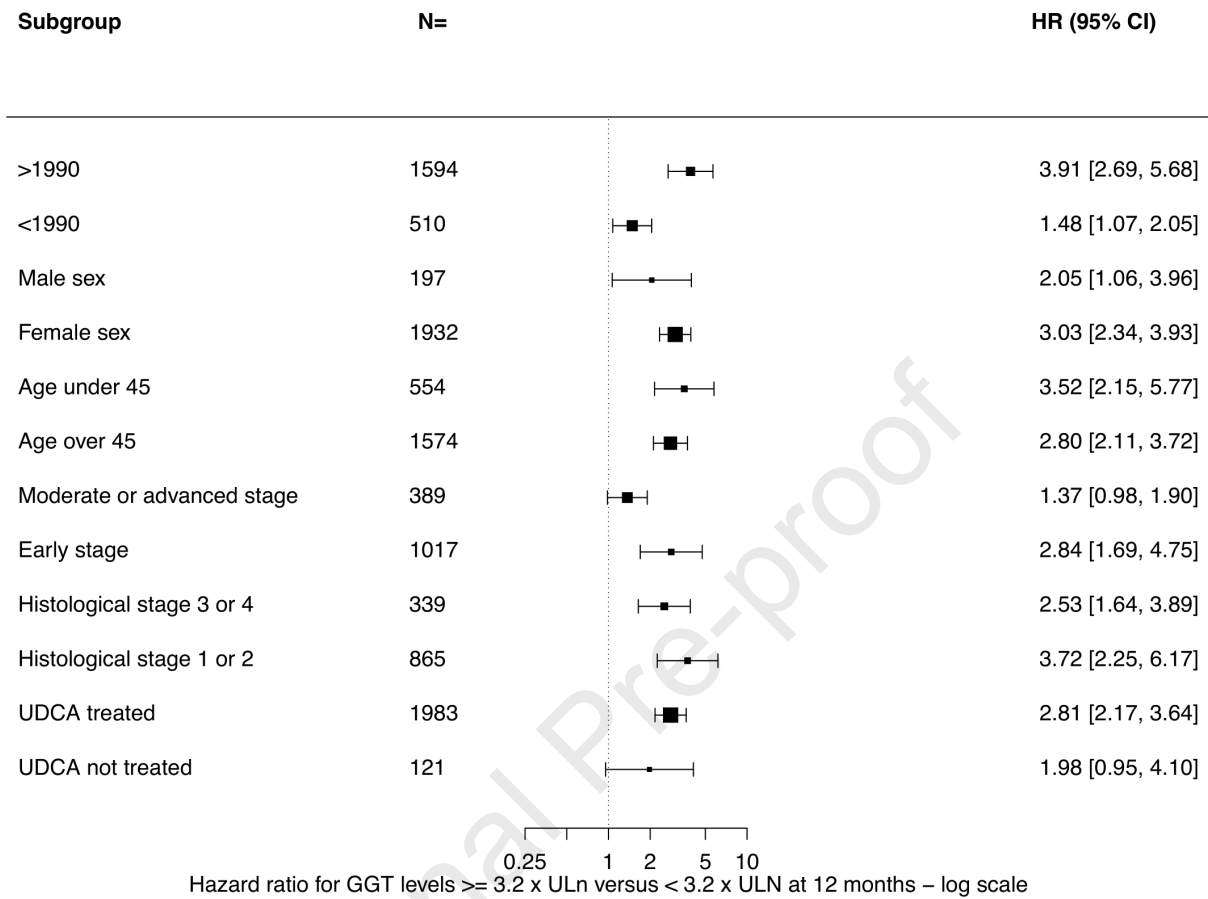
Factors	Multivariate model	
	HR (95% CI)	p
Age at diagnosis, years	1.01 (0.99,1.02)	0.08
Log ALP x ULN at 12 months	2.54 (1.27,5.05)	0.009
Log GGT x ULN at 12 months	2.55 (1.58,4.10)	0.00039
Bilirubin x ULN at 12 months	1.27 (1.21,1.33)	p < 0.0001
Albumin x ULN at 12 months (per 0.1)	0.66 (0.60,0.73)	p < 0.0001
Platelets (per 10)	0.93 (0.91,0.95)	p < 0.0001

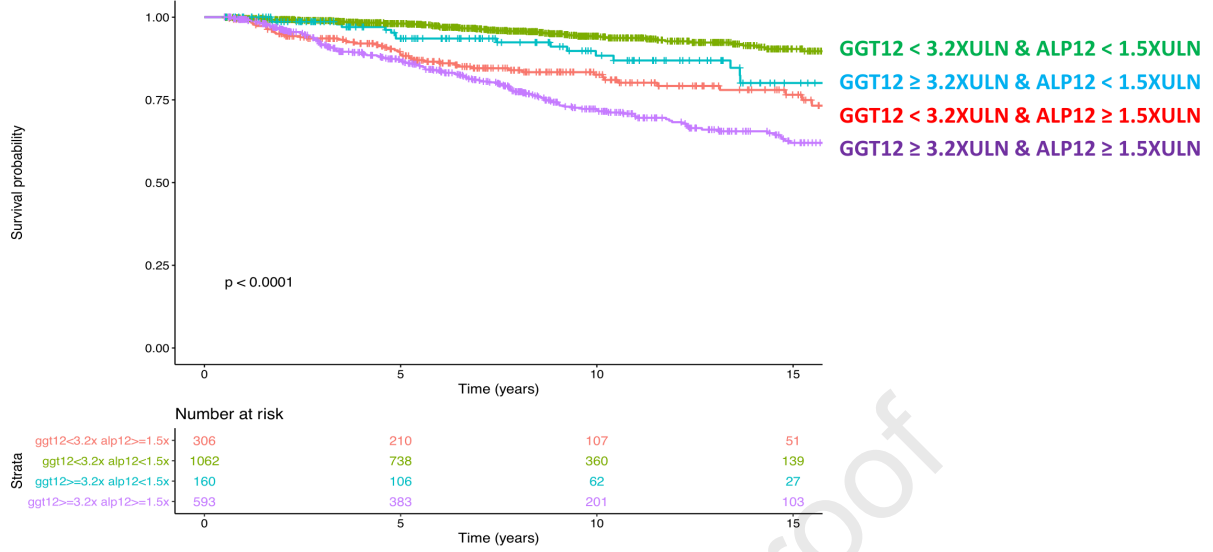
Note: the above analysis has been corrected stratifying by center.

Abbreviations: HR, hazard ratio; CI, confidence interval; p, p-value; GGT, gamma-glutamyl transferase.



A**B**





	Group 1	Group 2	Group 3
Group 1	/	0.006	1e-10
Group 2	0.006	/	0.12
Group 4	<math>< 2e-16</math>	0.006	0.009

Figure Legends

Figure 1. Correlation between levels of logGGT and logALP at 12 months. Spearman correlation $R=0.71$

Abbreviations: Log = logarithm; GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase. ULN = upper limit of normal.

Figure 2. The hazard ratio of liver transplantation or death for GGT levels at different time points (**A**, at baseline; **B**, at 12 months) with cubic spline transformation estimated by Cox regression.

Abbreviations: GGT = gamma-glutamyl transferase. ULN = upper limit of normal.

Figure 3. Subgroup analysis of GGT levels. HRs of liver transplantation or liver-related death for GGT levels ≥ 3.2 times the ULN versus < 3.2 times the ULN at 12-month follow-up for different subgroups. Early, moderate or advanced stage are defined according to Rotterdam criteria.

Abbreviations: GGT = gamma-glutamyl transferase. HRs = hazard ratios. ULN = upper limit of normal. UDCA = ursodeoxycholic acid.

Figure 4. Transplant-free survival of patients with GGT levels < 3.2 times the ULN versus ≥ 3.2 times the ULN at 12-month follow-up in both patients with ALP levels < 1.5 times the ULN and ≥ 1.5 times the ULN. Pairwise comparisons among the survival rates of the four groups have been estimated by log-rank test with Benjamini-Hochberg adjustment for multiplicity. Groups are defined as follows: **Group 1** GGT at 12 months $< 3.2 \times$ ULN & ALP

at 12 months $< 1.5 \times \text{ULN}$; **Group 2** GGT at 12 months $\geq 3.2 \times \text{ULN}$ & ALP at 12 months $< 1.5 \times \text{ULN}$; **Group 3** GGT at 12 months $< 3.2 \times \text{ULN}$ & ALP at 12 months $\geq 1.5 \times \text{ULN}$; **Group 4** GGT at 12 months $\geq 3.2 \times \text{ULN}$ & ALP at 12 months $\geq 1.5 \times \text{ULN}$.

Abbreviations: ULN = upper limit of normal, NS = not significant, GGT = gamma-glutamyl transferase, ALP = alkaline phosphatase.

Measurement of Gamma Glutamyl Transferase to Determine Risk of Liver Transplantation or Death in Patients With Primary Biliary Cholangitis

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on behalf of the Italian PBC Study Group and the GLOBAL PBC Study Group

Supplementary Material

Supplementary Tables.....2
Supplementary Figures6

Supplementary Tables

Supplementary Table 1 Features of excluded cases compared to included ones

		Included	Excluded ^a	p-value
		(N = 2129)	(N=2116)	
Age at diagnosis		53.1± 12.1	51.9±11.7	1.00
Year of diagnosis	< 1990	510 (24.2)	490 (24.4)	0.95
	>1990	1594 (75.8)	1521 (75.6)	
Female sex (%)		1932 (90.7)	1913 (90.4)	0.74
AMA positive (%)		1888 (89.1)	1852 (89.0)	0.90
Treated with UDCA (%)	1	1983 (94.2)	1782 (87.2)	<0.001
Histological disease stage^b (%)	0	0 (0.0)	2 (0.2)	<0.001
	1	533 (44.3)	307 (28.4)	
	2	332 (27.6)	364 (33.6)	
	3	221 (18.4)	154 (14.2)	
	4	118 (9.8)	255 (23.6)	
Biochemical disease stage^c (%)	early	1017 (72.3)	701 (64.3)	0.30
	moderately	313 (22.3)	302 (27.7)	
	advanced	76 (5.4)	302 (27.7)	
Serum GGT at baseline (x ULN)		5.98 [3.12, 10.88]	6.56 [3.03, 11.62]	0.36
Serum ALP at baseline (x ULN)		2.16 [1.34, 3.80]	2.27 [1.37, 4.10]	0.12
Serum bilirubin at baseline (x ULN)		0.6 [0.45,1.00]	0.64 [0.43, 1.04]	0.90
Serum AST at baseline (x ULN)		1.5 [1.00-2.24]	1.53 [1.00, 2.46]	0.23
Serum ALT at baseline (x ULN)		1.6 [1.00-2.54]	1.68 [1.07, 2.75]	0.14
Serum ALP at 12 months (x ULN)		1.30 [0.89, 2.15]	1.42 [0.94, 2.44]	0.20
Serum bilirubin at 12 months (x ULN)		0.58 [0.41, 0.83]	0.55 [0.38, 0.86]	0.36
Serum AST at 12 months (x ULN)		0.93 [0.70, 1.41]	0.55 [0.38, 0.86]	0.69
Serum ALT at 12 months (x ULN)		0.89 [0.60, 1.50]	0.90 [0.60, 1.56]	0.14
Serum albumin at 12 months (x LLN)		1.16 [1.08, 1.25]	1.15 [1.06, 1.26]	0.52
Median follow up (years)		7.50 [3.91, 12.18]	7.79 [4.13, 11.96]	0.42

^aexcluded patients are those without GGT at 12 months or with GGT ratio ≤ 0.2 among 4245 patients available for analysis

^bhistological disease stage according to Ludwig and Scheuer's classification

°biochemical disease stage according to Rotterdam criteria (using albumin and bilirubin)

AMA= anti mitochondrial antibodies; UDCA = ursodeoxycholic acid; GGT = gamma-glutamyl transferase;

ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

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Supplementary Table 2 Missing values

		Missings (%)
Age at diagnosis		1 (0.5)
Year of diagnosis		25 (1.2)
Female (%)		/
AMA positive (%)		9 (0.4)
Treated with UDCA (%)		25 (1.2)
Histological disease stage ^a (%)		925 (43.4)
Biochemical disease stage ^b (%)		723 (34.0)
Serum GGT at baseline (x ULN)		279 (13.1)
Serum ALP at baseline (x ULN)		387 (18.2)
Serum bilirubin at baseline (x ULN)		389 (18.3)
Serum AST at baseline (x ULN)		300 (14.1)
Serum ALT at baseline (x ULN)		300 (14.1)
Serum GGT at 12 months (x ULN)		/
Serum ALP at 12 months (x ULN)		7 (0.3)
Serum bilirubin at 12 months (x ULN)		205 (9.6)
Serum albumin at 12 months (x LLN)		660 (31.0)
Serum AST at 12 months (x ULN)		54 (2.5)
Serum ALT at 12 months (x ULN)		42 (2.0)

AMA= anti mitochondrial antibodies; UDCA = ursodeoxicholic acid; GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine amino transferase.

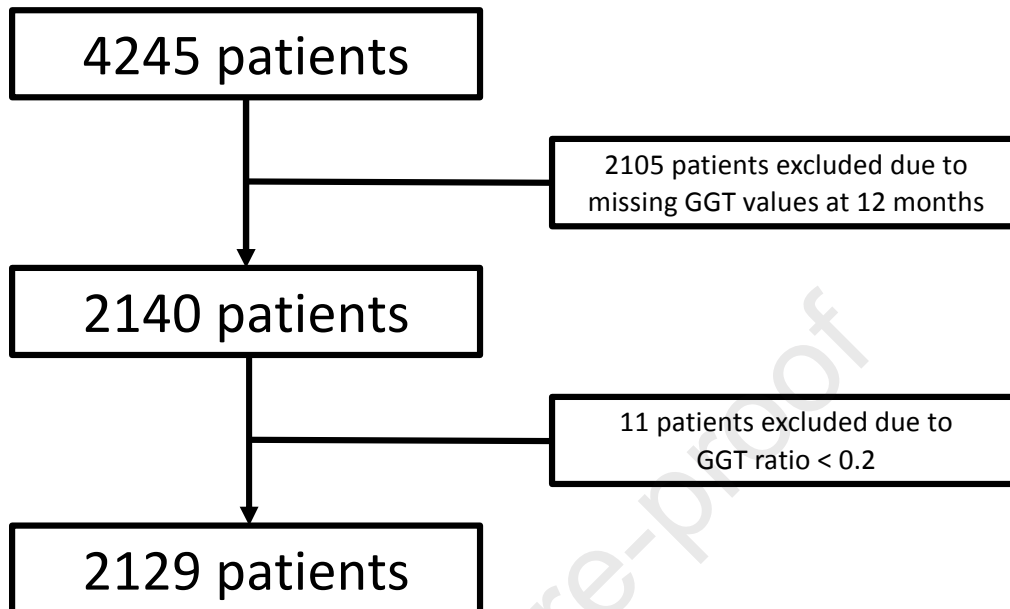
Supplementary Table 3 Comparison between patients with isolated elevated GGT and patients with GGT and ALP under threshold

		GGT₁₂<3.2 & ALP₁₂<1.5	GGT₁₂≥3.2 & ALP₁₂<1.5
n		1063	160
Age at diagnosis		54.6 (46.4, 63.0)	52.6 (44.2, 61.9)
Year of diagnosis	< 1990	133 (12.7)	38 (23.8)
	> 2000	911 (87.3)	122 (76.2)
Female sex (%)		963 (90.6)	135 (84.4)
AMA positive (%)		943 (89.1)	141 (88.1)
Treated with UDCA (%)		1016 (96.7)	145 (91.8)
Histological stage ^a	1	326 (52.1)	42 (48.3)
	2	170 (27.2)	21 (24.1)
	3	85 (13.6)	19 (21.8)
	4	45 (7.2)	5 (5.7)
Serum total bilirubin at 12 months (x ULN)		0.50 (0.38, 0.67)	0.53 (0.40, 0.71)
Serum albumin at 12 months (x ULN)		1.18 (1.10, 1.26)	1.18 (1.09, 1.25)
Serum ALP at 12 months (x ULN)		0.91 (0.70, 1.14)	1.19 (0.99, 1.31)
Serum AST at 12 months (x ULN)		0.74 (0.60, 0.94)	0.93 (0.77, 1.17)
Serum ALT at 12 months (x ULN)		0.66 (0.49, 0.90)	0.91 (0.72, 1.35)
Serum GGT at 12 months (x ULN)		1.06 (0.67, 1.75)	4.50 (3.68, 6.07)

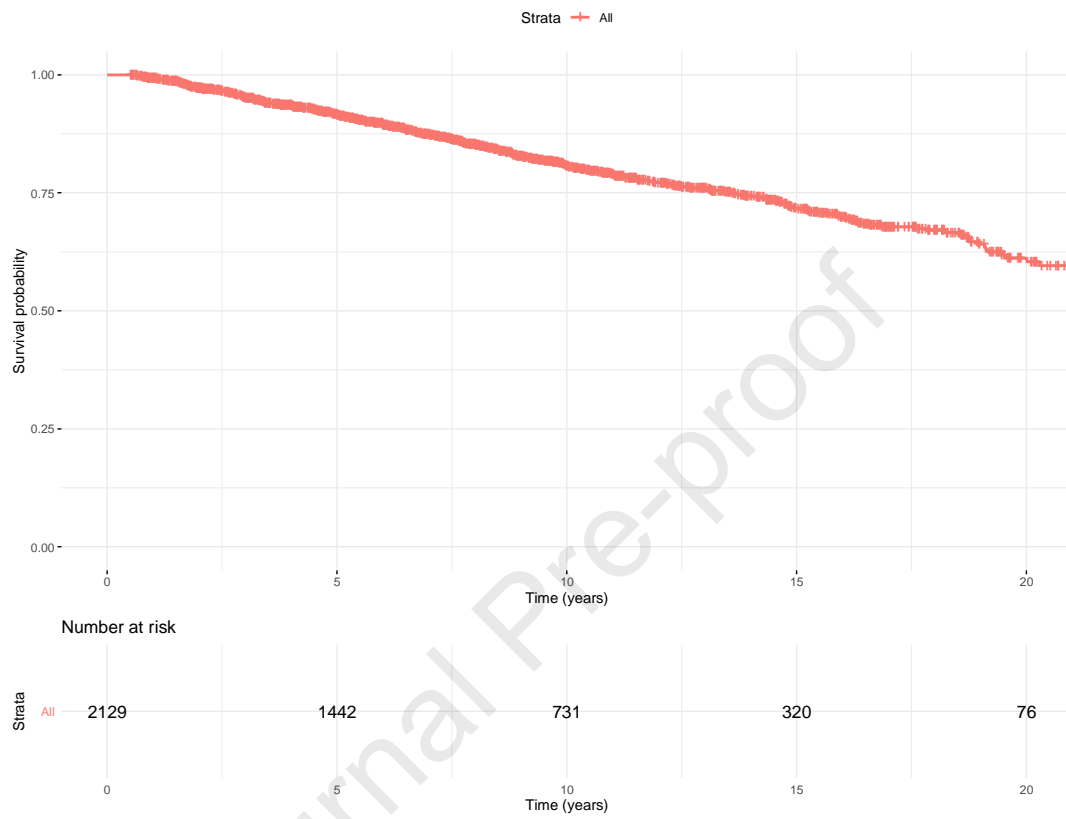
^ahistological disease stage according to Ludwig and Scheuer's classification

AMA= anti mitochondrial antibodies; UDCA = ursodeoxicholic acid; GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine amino transferase.

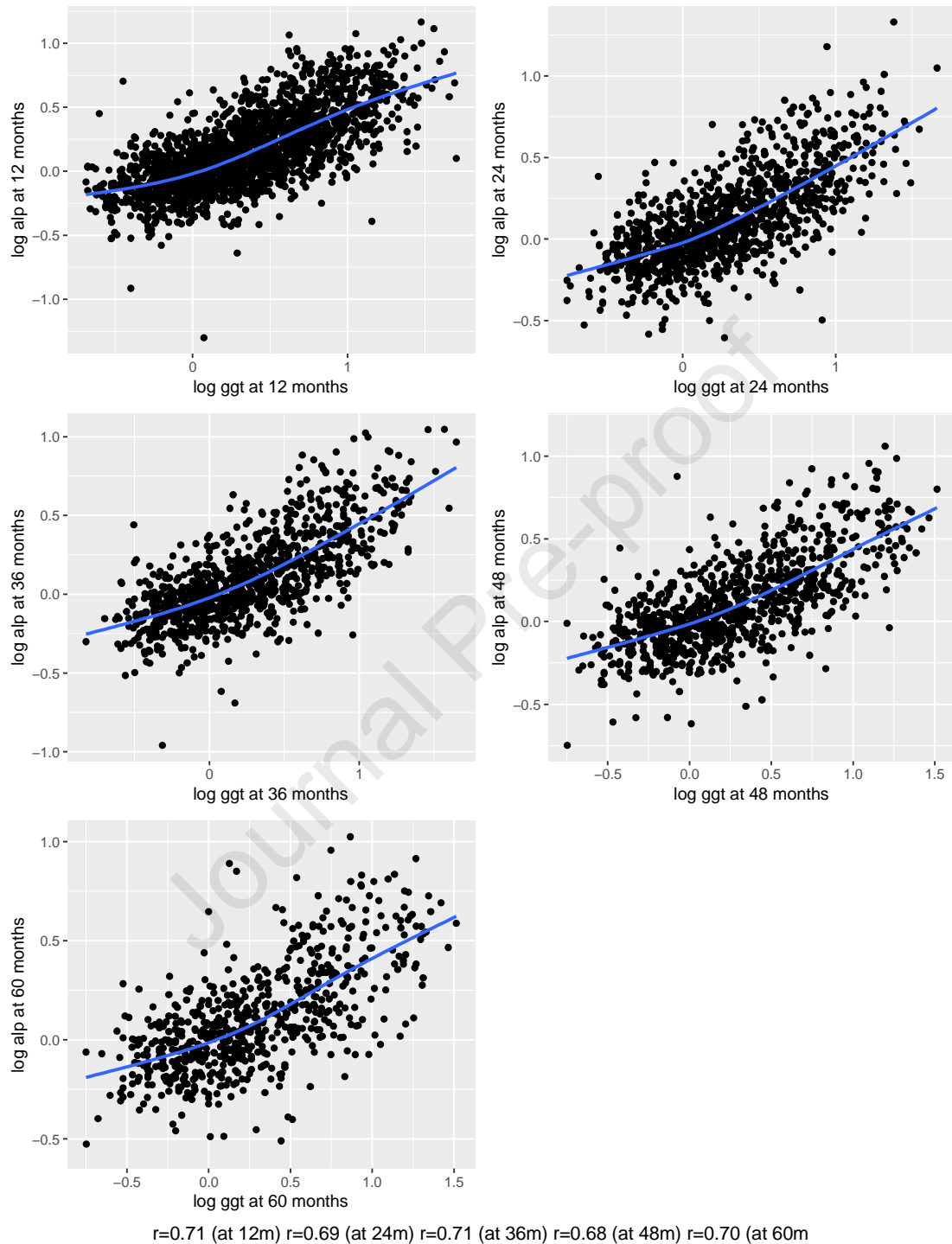
Supplementary Figures



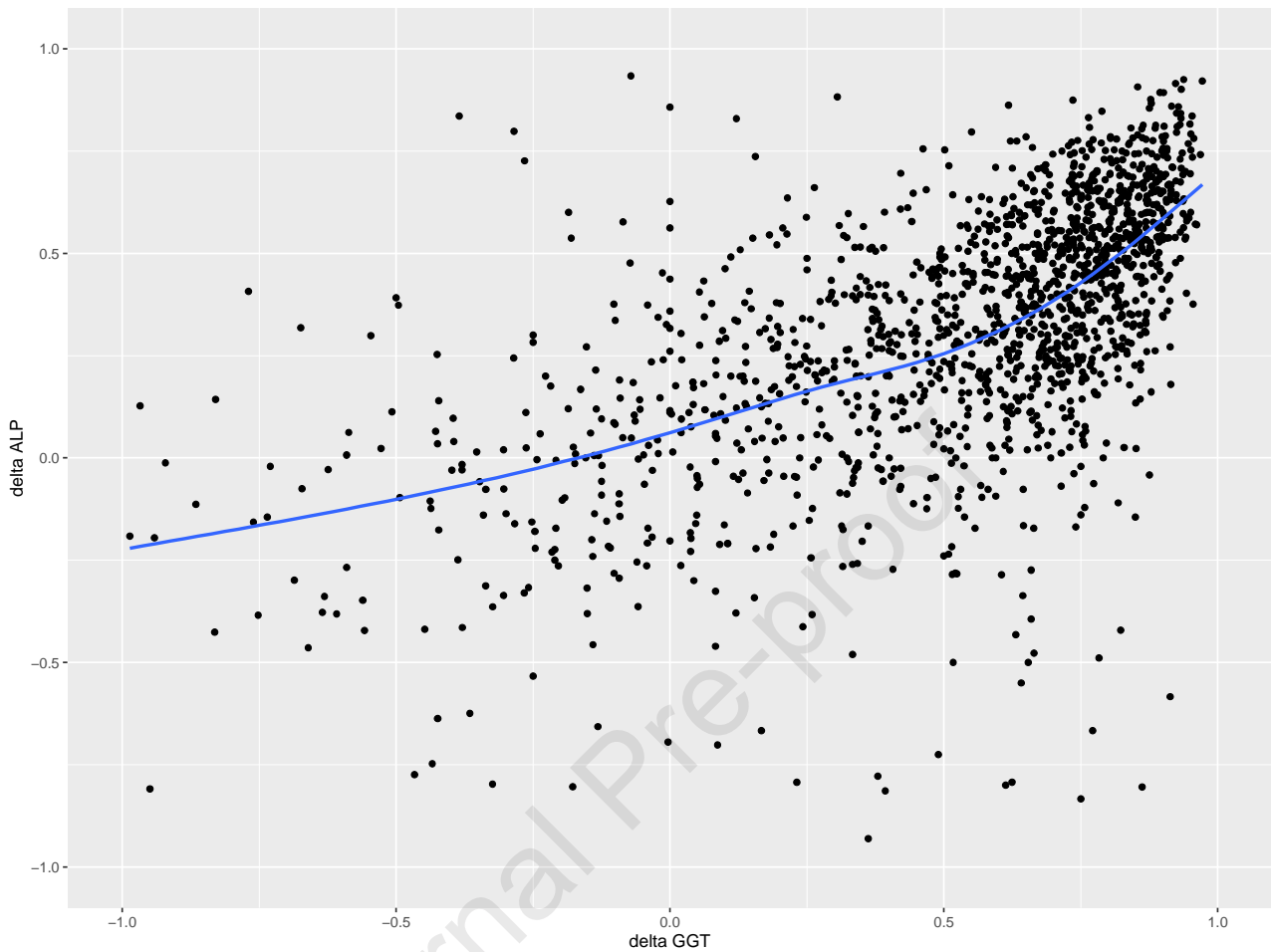
Supplementary Figure 1. Case selection



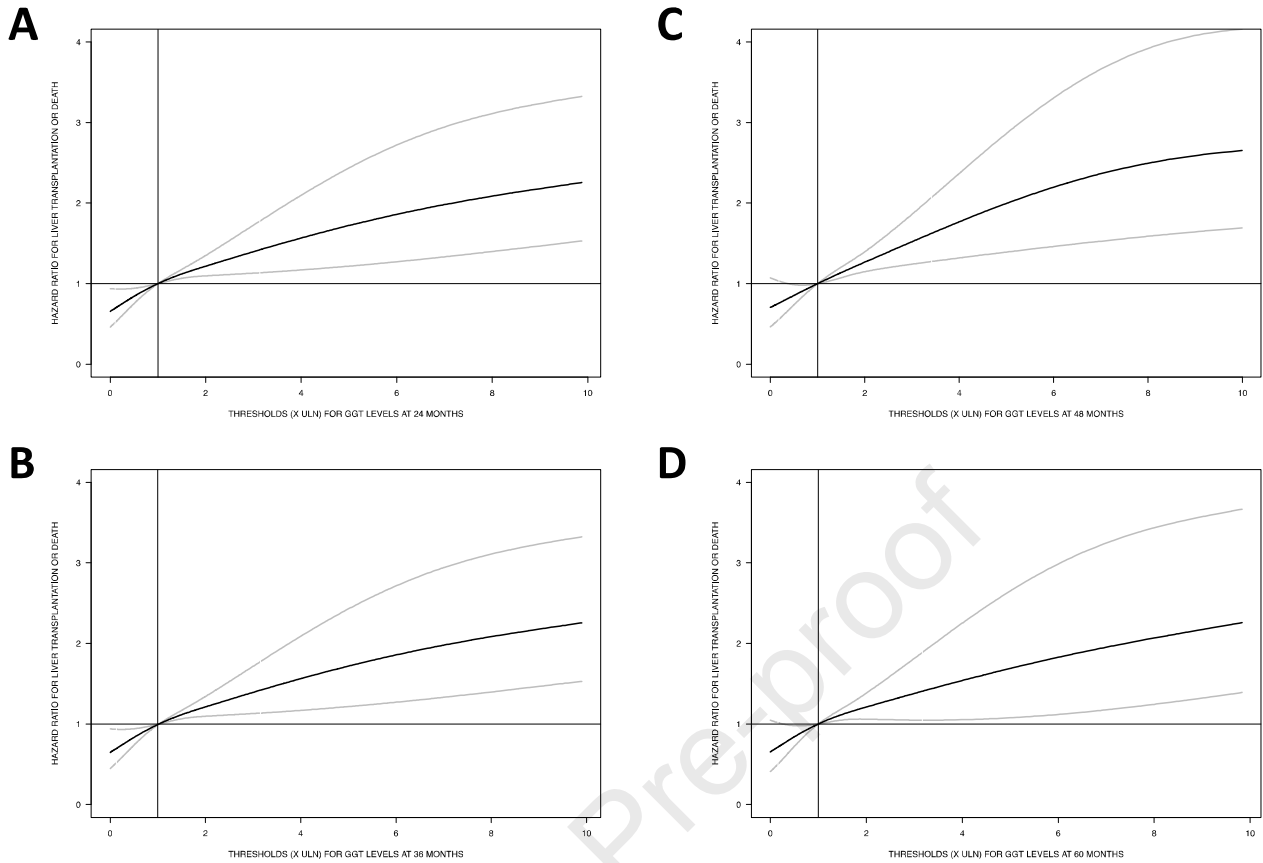
Supplementary Figure 2. Transplant-free survival for the overall cohort.



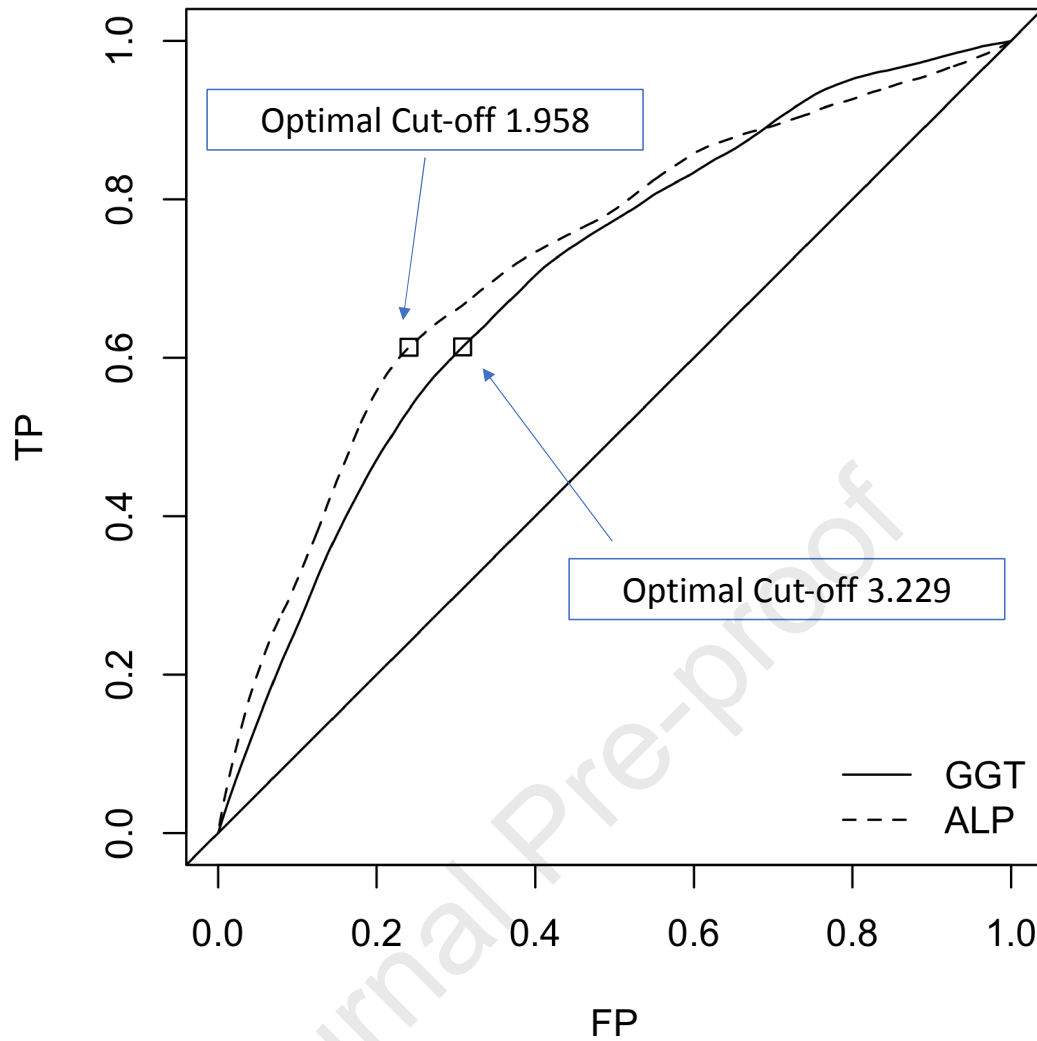
Supplementary Figure 3. Correlation between levels of log GGT and log ALP at different time points (up to 5-year follow-up). Log = logarithm; GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase.



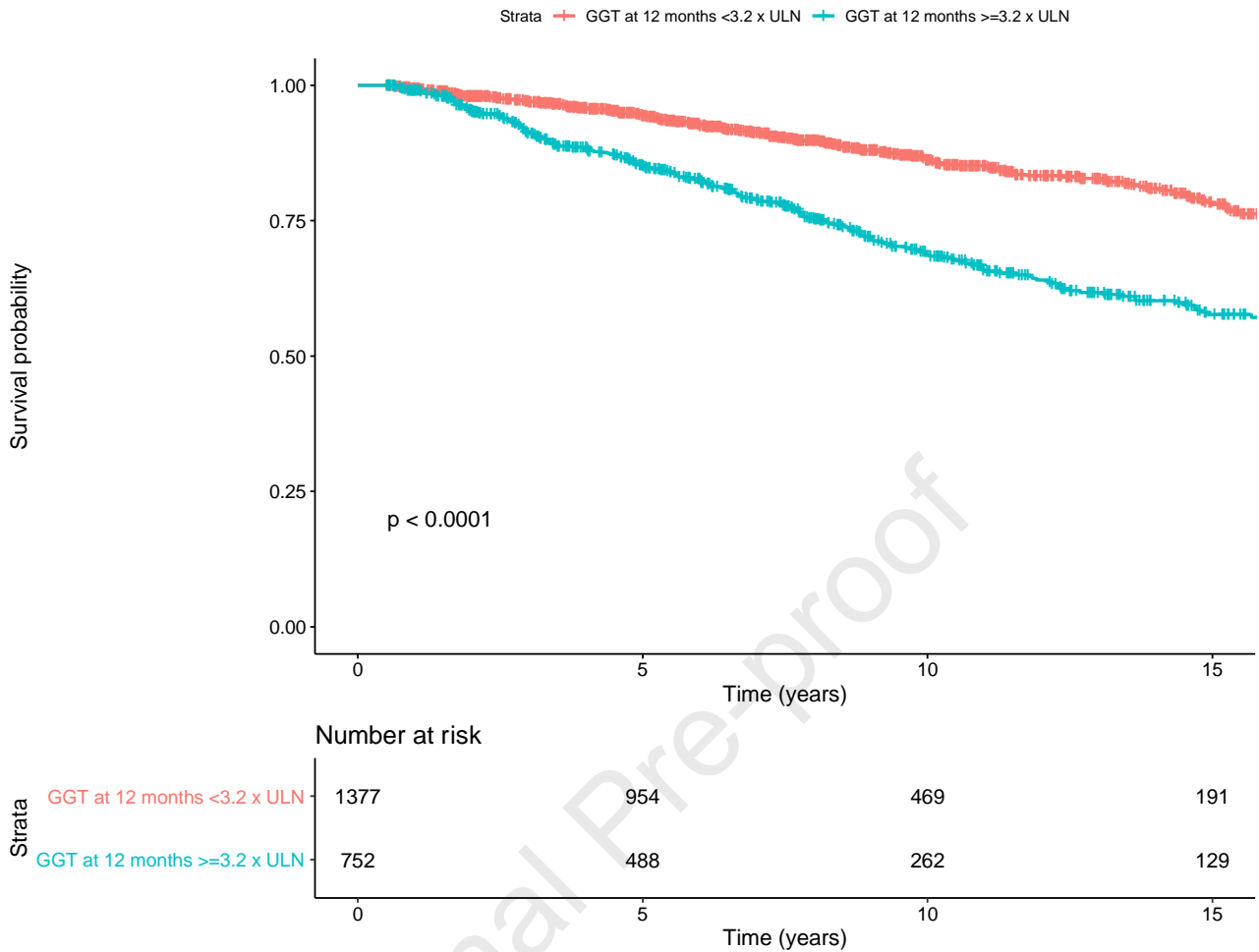
Supplementary Figure 4. Correlation between levels of delta GGT and delta ALP in patients treated with UDCA (n=1630) . Delta GGT is $(\text{GGT at baseline} - \text{GGT at 12 months})/\text{GGT at baseline}$. Delta ALP is $(\text{ALP at baseline} - \text{ALP at 12 months})/\text{ALP at baseline}$. GGT = gamma-glutamyl transferase, ALP = alkaline phosphatase, UDCA = ursodeoxycholic acid. Spearman correlation $R=0.65$.



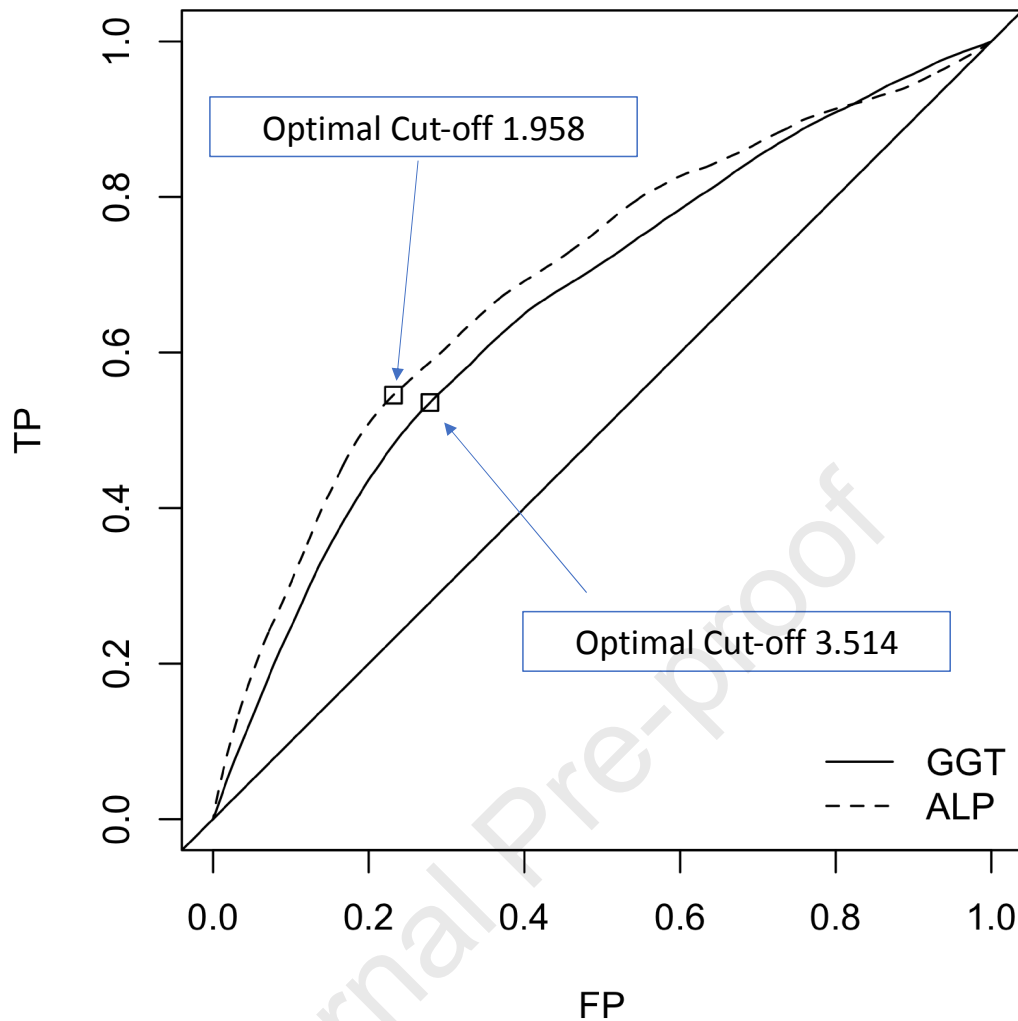
Supplementary Figure 5. The hazard ratio of liver transplantation or death for GGT levels at different time points (**A**, at 24 months; **B**, at 36 months; **C**, at 48 months; **D**, at 60 months) with cubic spline transformation estimated by Cox regression. GGT = gamma-glutamyl transferase, ULN = upper limit of normal.



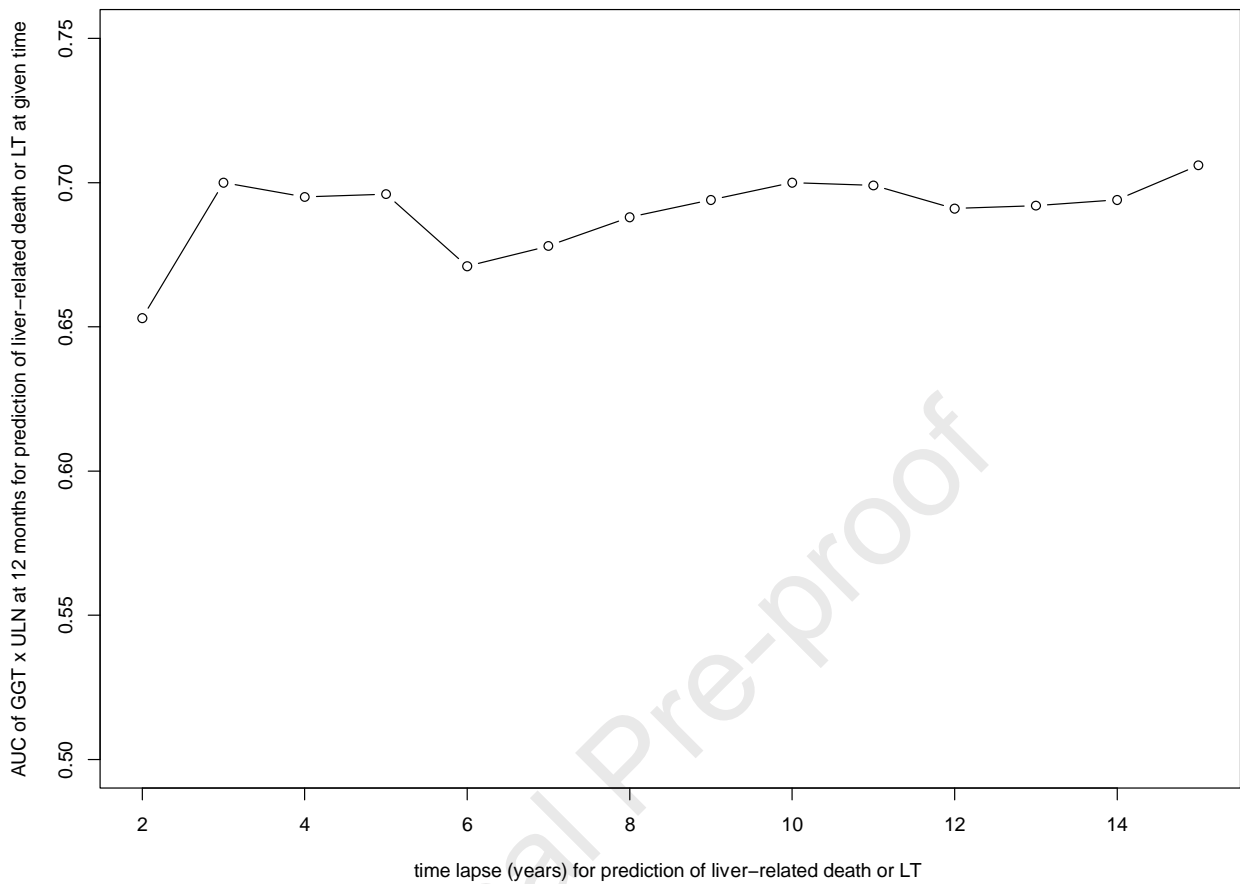
Supplementary Figure 6. Time-dependent ROC curve analysis for prediction of liver-related death or liver transplantation at 10 years based on levels of GGT and ALP at 12 months of follow-up. AUROC for GGT = 0.70, AUROC for ALP 0.724. ROC = receiver operating characteristic; AUROC = area under the ROC curve; FP = false positive; TP = true positive; GGT = gamma-glutamyl transferase ALP = alkaline phosphatase.



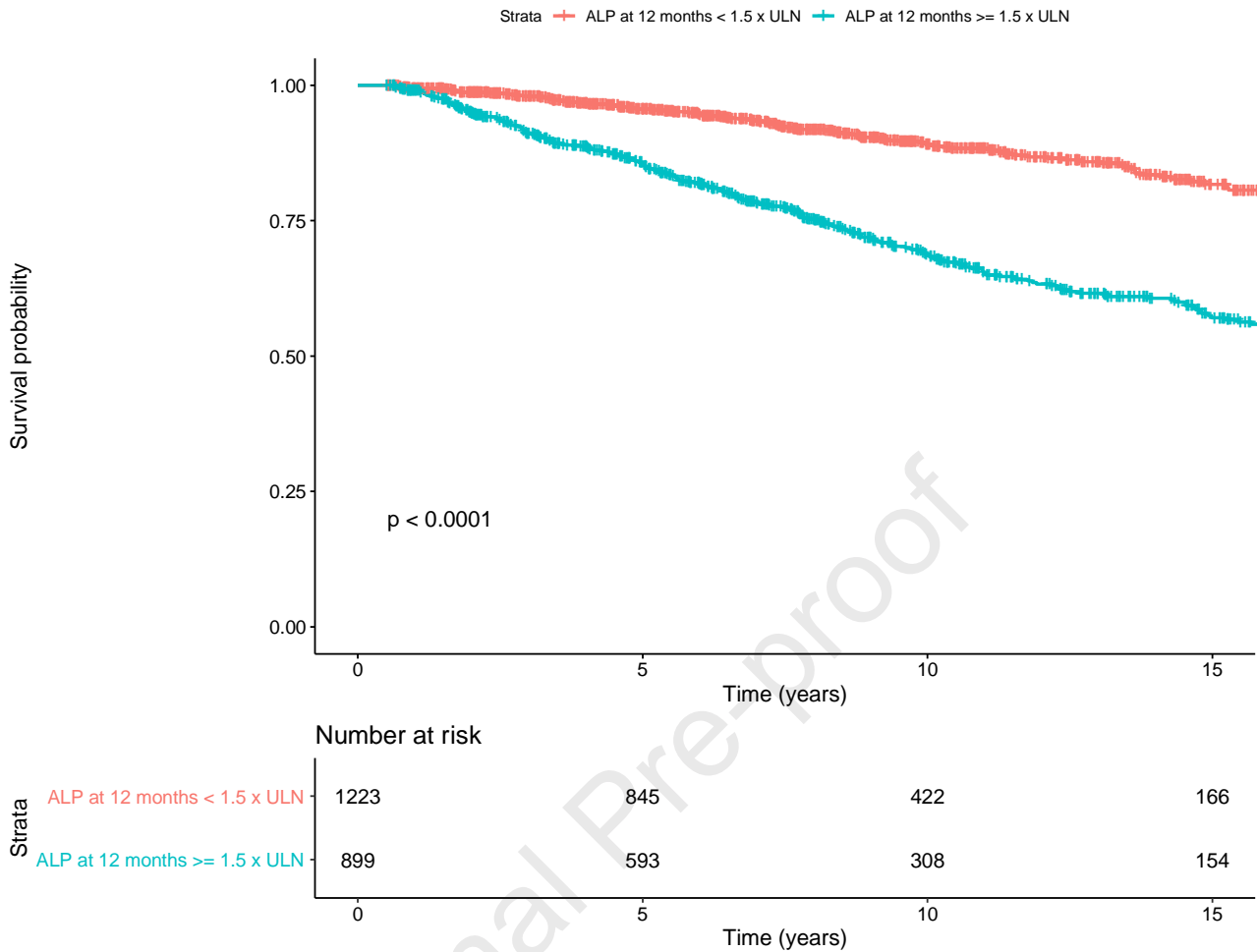
Supplementary Figure 7. Transplant-free survival of patients with GGT levels < 3.2 times the ULN versus \geq 3.2 times the ULN at 12-month follow-up. GGT = gamma-glutamyl transferase. ULN = upper limit of normal.



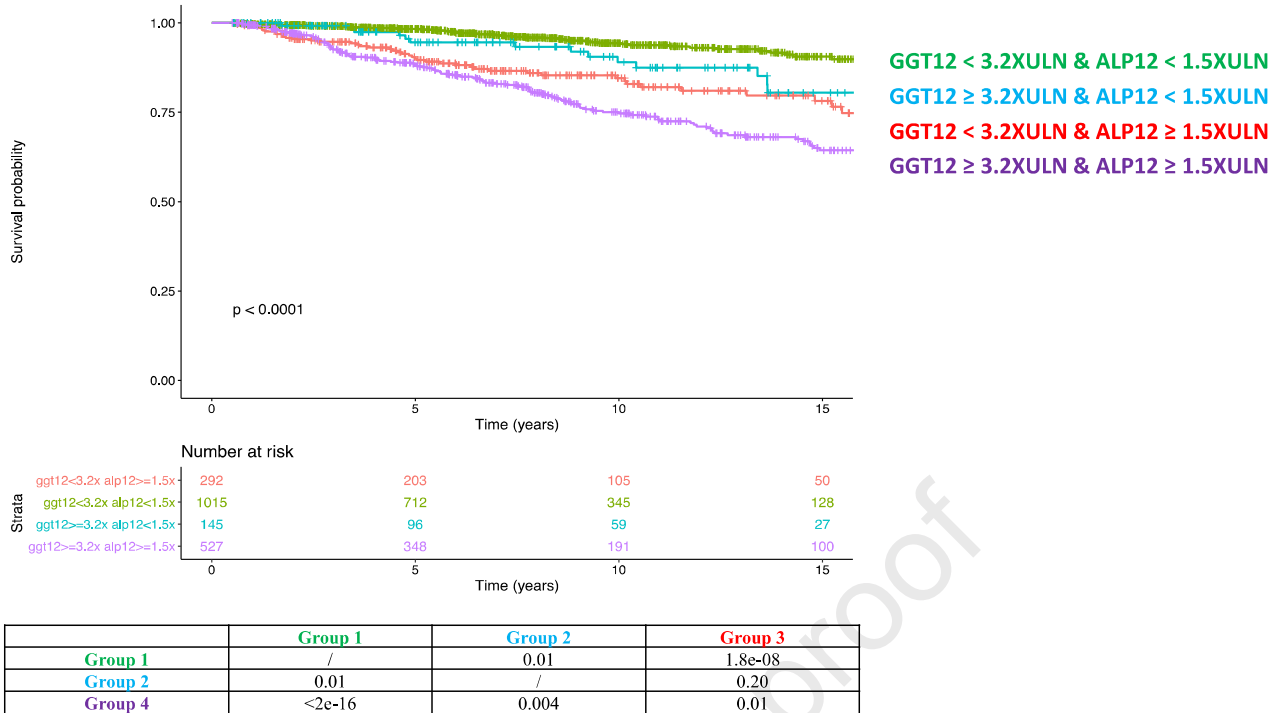
Supplementary Figure 8. Time-dependent ROC curve analysis for prediction of all-cause death or liver transplantation at 10 years based on levels of GGT and ALP at 12 months of follow-up. AUROC for GGT = 0.663, AUROC for ALP 0.697. ROC = receiver operating characteristic; AUROC = area under the ROC curve; FP = false positive; TP = true positive; GGT = gamma-glutamyl transferase ALP = alkaline phosphatase.



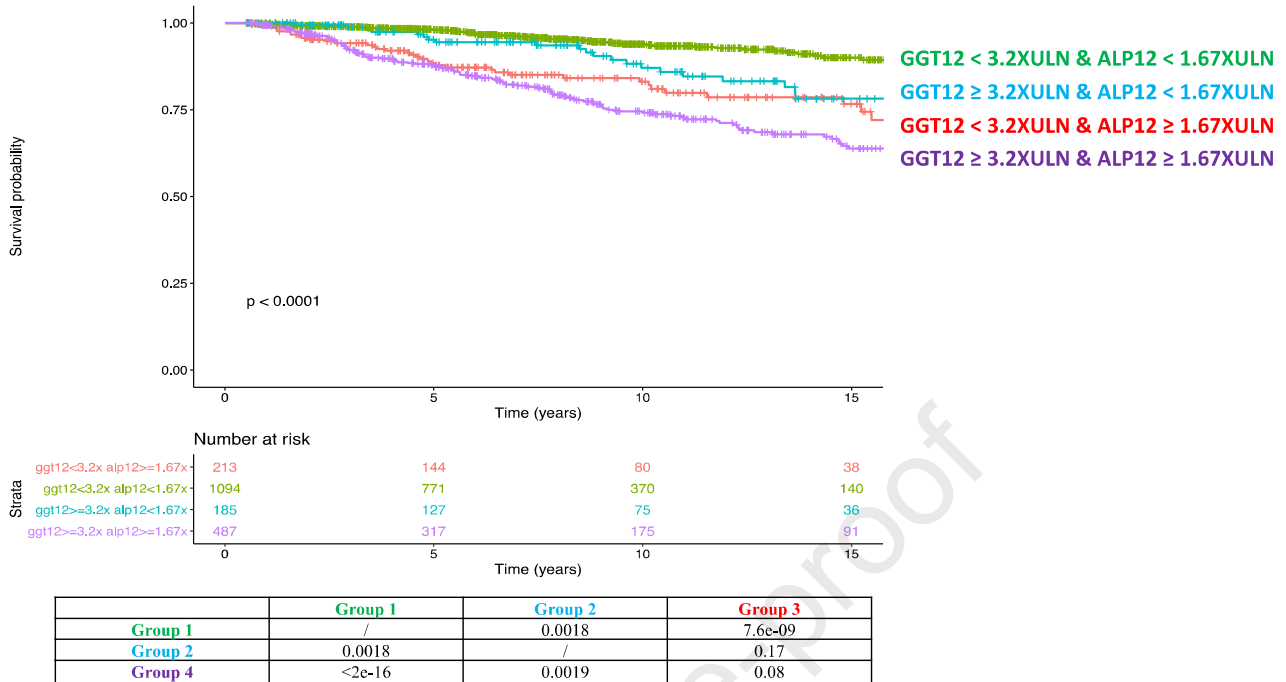
Supplementary Figure 9. Trend of the AUC values, calculated by time-dependent ROC curve analysis, of GGT at 12 months of follow-up for prediction of liver-related death or liver transplantation at different time points. AUC = area under the curve ; GGT = gamma-glutamyl transferase; ULN = upper limit of normal; LT = liver transplantation.



Supplementary Figure 10. Transplant-free survival of patients with ALP levels < 1.5 times the ULN versus \geq 1.5 times the ULN at 12-month follow-up. ALP = alkaline phosphatase. ULN = upper limit of normal.

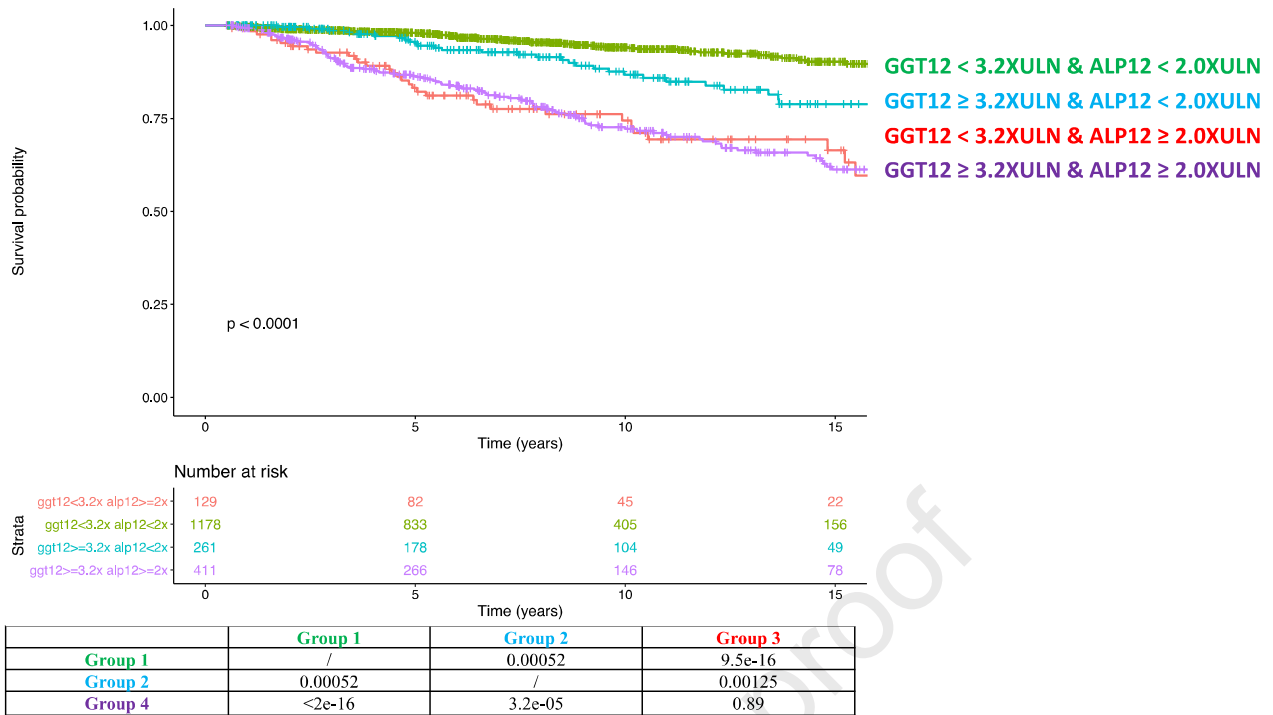


Supplementary Figure 11. Transplant-free survival of patients with GGT levels < 3.2 times the ULN versus ≥ 3.2 times the ULN at 12-month follow-up in both patients with ALP levels < 1.5 times the ULN and ≥ 1.5 times the ULN, in the sub-cohort of UDCA treated patients ($n = 1983$). GGT = gamma-glutamyl transferase. Pairwise comparisons among the survival rates of the four groups (including only UDCA-treated patients) have been estimated by log-rank test with Benjamini-Hochberg adjustment for multiplicity. Groups are defined as follows: **Group 1** GGT at 12 months < 3.2 x ULN & ALP at 12 months < 1.5 x ULN; **Group 2** GGT at 12 months ≥ 3.2 x ULN & ALP at 12 months < 1.5 x ULN; **Group 3** GGT at 12 months < 3.2 x ULN & ALP at 12 months ≥ 1.5 x ULN; **Group 4** GGT at 12 months ≥ 3.2 x ULN & ALP at 12 months ≥ 1.5 x ULN. ULN = upper limit of normal. UDCA = ursodeoxicholic acid; GGT = gamma-glutamyl transferase, ALP = alkaline phosphatase.



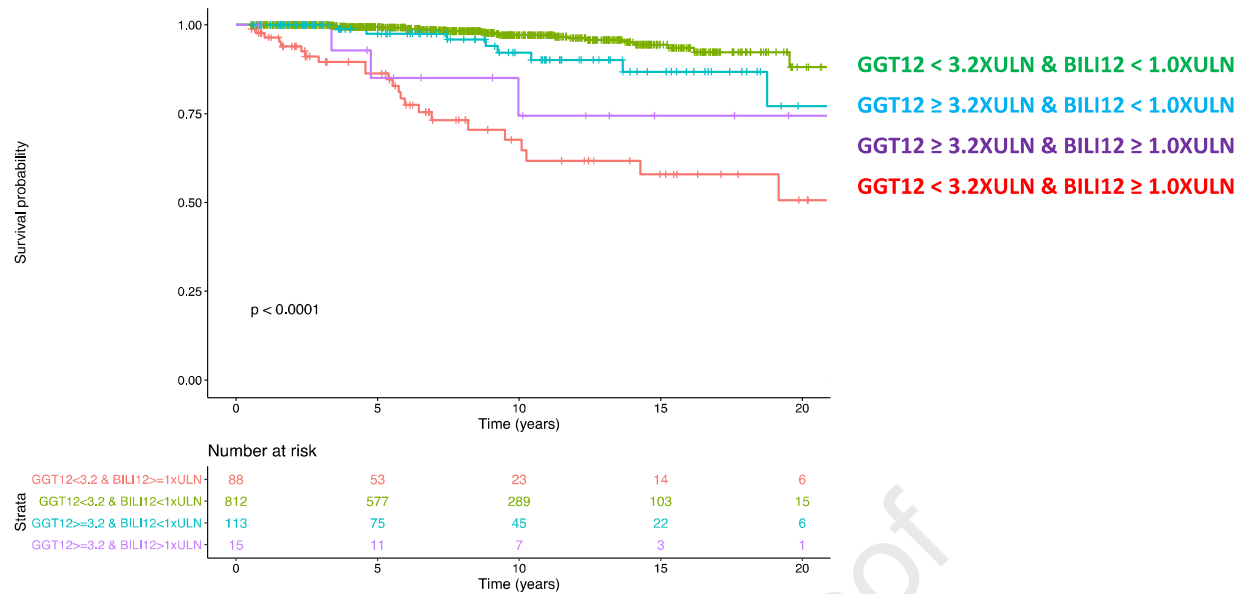
Supplementary Figure 12. Transplant-free survival of patients with GGT levels < 3.2 times the ULN versus ≥ 3.2 times the ULN at 12-month follow-up in both patients with ALP levels < 1.67 times the ULN and ≥ 1.67 times the ULN, in the sub-cohort of UDCA treated patients ($n = 1983$). Pairwise comparisons among the survival rates of the four groups have been estimated by log-rank test with Benjamini-Hochberg adjustment for multiplicity. Groups are defined as follows: **Group 1** GGT at 12 months $< 3.2 \times$ ULN & ALP at 12 months $< 1.67 \times$ ULN; **Group 2** GGT at 12 months $\geq 3.2 \times$ ULN & ALP at 12 months $< 1.67 \times$ ULN; **Group 3** GGT at 12 months $< 3.2 \times$ ULN & ALP at 12 months $\geq 1.67 \times$ ULN; **Group 4** GGT at 12 months $\geq 3.2 \times$ ULN & ALP at 12 months $\geq 1.67 \times$ ULN.

Abbreviations: ULN = upper limit of normal, NS = not significant, GGT = gamma-glutamyl transferase, ALP = alkaline phosphatase.



Supplementary Figure 13. Transplant-free survival of patients with GGT levels < 3.2 times the ULN versus ≥ 3.2 times the ULN at 12-month follow-up in both patients with ALP levels < 2.0 times the ULN and ≥ 2.0 times the ULN, in the sub-cohort of UDCA treated patients ($n = 1983$). Pairwise comparisons among the survival rates of the four groups have been estimated by log-rank test with Benjamini-Hochberg adjustment for multiplicity. Groups are defined as follows: **Group 1** GGT at 12 months < 3.2 x ULN & ALP at 12 months < 2.0 x ULN; **Group 2** GGT at 12 months ≥ 3.2 x ULN & ALP at 12 months < 2.0 x ULN; **Group 3** GGT at 12 months < 3.2 x ULN & ALP at 12 months ≥ 2.0 x ULN; **Group 4** GGT at 12 months ≥ 3.2 x ULN & ALP at 12 months ≥ 2.0 x ULN.

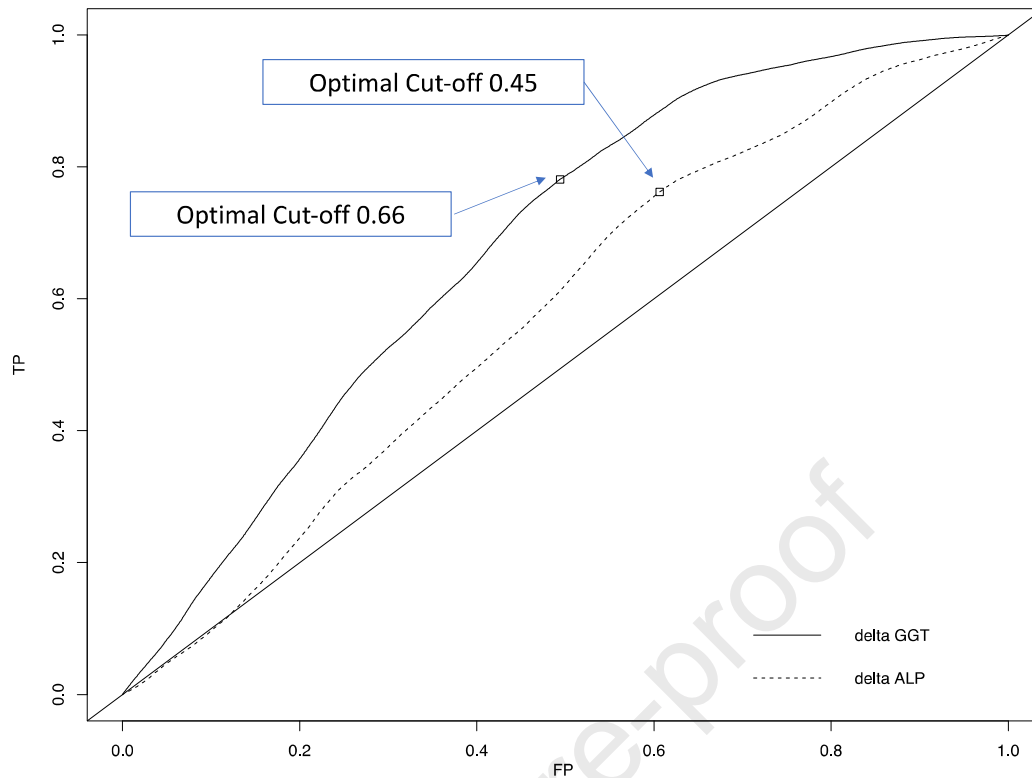
Abbreviations: ULN = upper limit of normal, NS = not significant, GGT = gamma-glutamyl transferase, ALP = alkaline phosphatase.



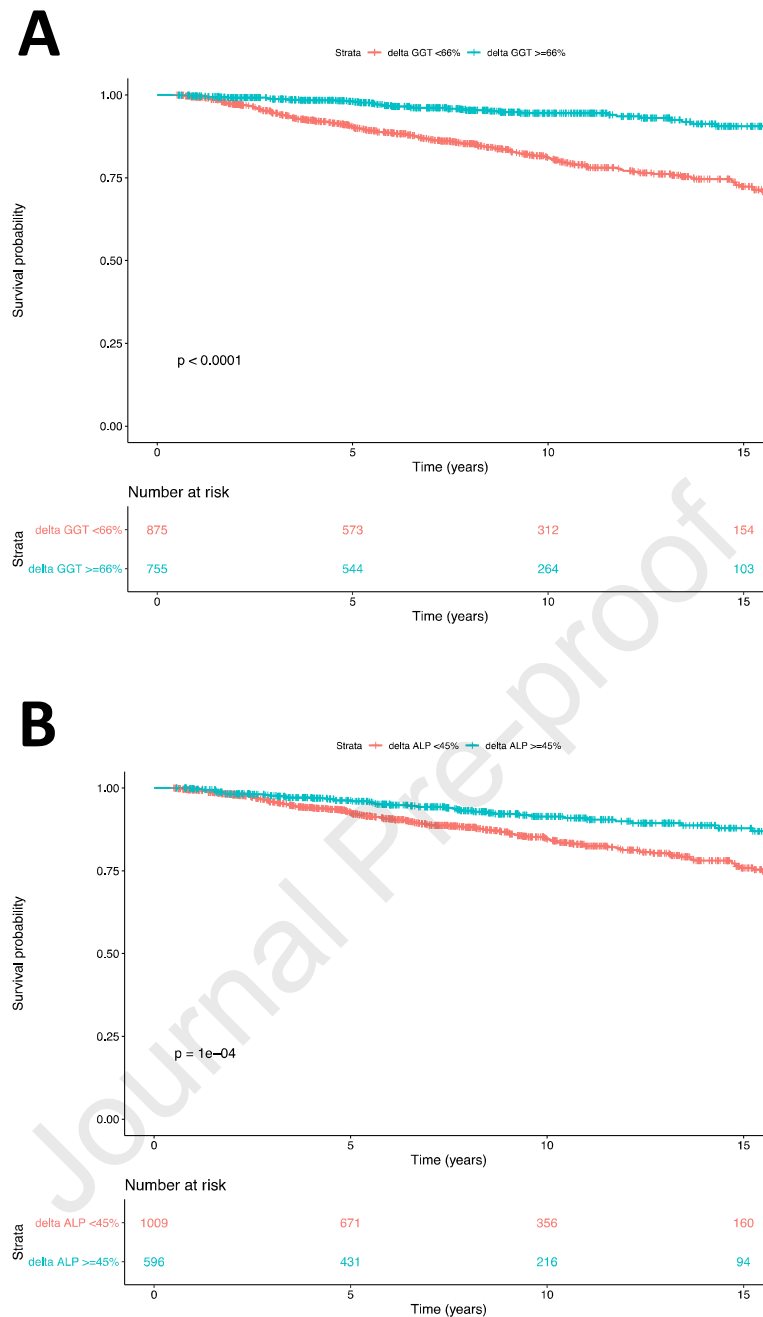
	Group 1	Group 2	Group 3
Group 1	/	0.029	<math>< 2e-16</math>
Group 2	0.029	/	0.00011
Group 4	0.00011	0.06	0.56

Supplementary Figure 14. Transplant-free survival of patients with GGT levels < 3.2 times the ULN versus ≥ 3.2 times the ULN at 12-month follow-up in both patients with total bilirubin levels < 1.0 times the ULN and ≥ 1.0 times the ULN, in the sub-cohort of UDCA treated patients with ALP levels $< 1.5 \times$ ULN ($n = 1161$). Pairwise comparisons among the survival rates of the four groups have been estimated by log-rank test with Benjamini-Hochberg adjustment for multiplicity. Groups are defined as follows: **Group 1** GGT at 12 months $< 3.2 \times$ ULN & BILI at 12 months $\geq 1.0 \times$ ULN; **Group 2** GGT at 12 months $< 3.2 \times$ ULN & BILI at 12 months $< 1.0 \times$ ULN; **Group 3** GGT at 12 months $\geq 3.2 \times$ ULN & BILI at 12 months $< 1.0 \times$ ULN; **Group 4** GGT at 12 months $\geq 3.2 \times$ ULN & BILI at 12 months $\geq 1.0 \times$ ULN.

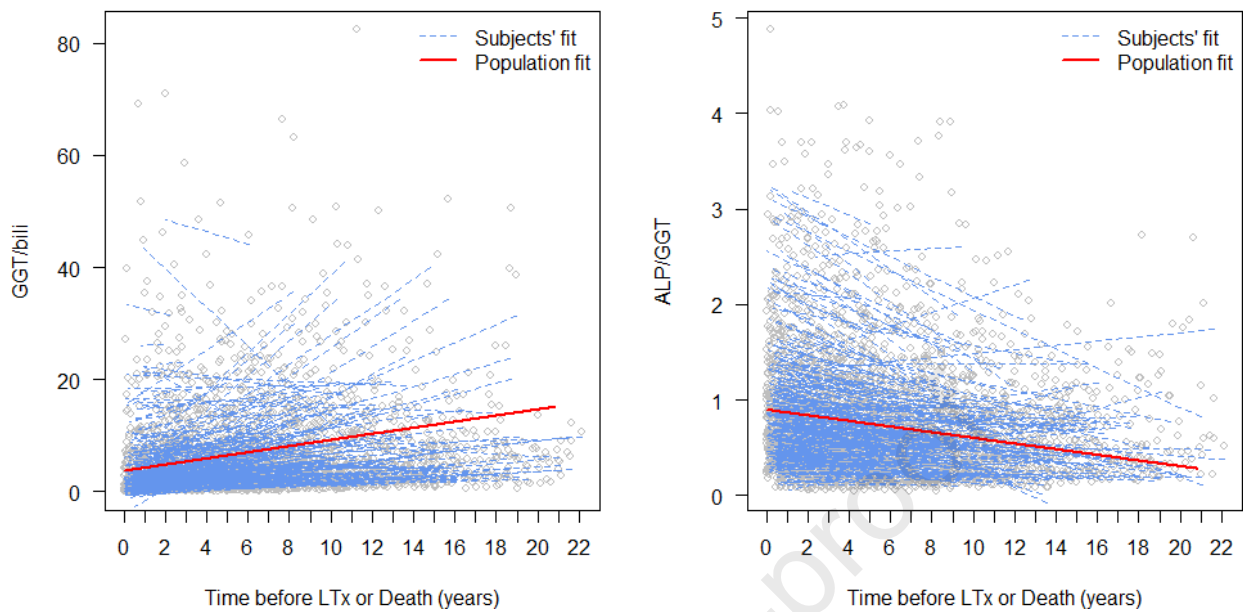
Abbreviations: ULN = upper limit of normal, GGT = gamma-glutamyl transferase, BILI = total bilirubin.



Supplementary Figure 15. Time-dependent ROC curve analysis for prediction of liver-related death or liver transplantation at 10 years based on levels of delta GGT and delta ALP. AUROC for delta GGT = 0.68, AUROC for delta ALP 0.58. ROC = receiver operating characteristic; AUROC = Area under the ROC curve; FP = false positive; TP = true positive; GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase.



Supplementary Figure 16. Transplant-free survival of patients with delta GGT < 0.66 times versus delta GGT ≥ 0.66 (A). Transplant-free survival of patients with delta ALP < 0.45 times versus delta ALP ≥ 0.45 (B). GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase. ULN = upper limit of normal.



Supplementary Figure 17. Behavioral pattern of GGT/bilirubin (A) and ALP/GGT (B) ratios over the disease course. Follow-up time on x-axis is reversed, and x-axis=0 corresponds to time of occurrence of the composite endpoint. Patients alive at last follow-up or censored are excluded from the analysis. GGT = gamma-glutamyl transferase, ALP = alkaline phosphatase; LTx = liver transplantation.

Need to Know

Background: Gamma-glutamyltransferase (GGT) is a serum marker of cholestasis, but it was not clear whether serum level of GGT is a prognostic marker for patients with primary biliary cholangitis (PBC).

Findings: Serum level of GGT can be used to identify patients with PBC at risk for liver transplantation or death, and increase the prognostic value of ALP measurement.

Implications for patient care: The findings support the use of GGT as primary clinical endpoint in clinical trials. In patients with low serum level of ALP, a high level of GGT identifies those who might require treatment of metabolic disorders or treatment escalation.