

PD-L1 and the risk of bacterial infection in patients with chronic liver diseases: An international multicohort study

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Table of contents

Supplementary figures.....	2
Supplementary tables.....	11

Supplementary figures

Fig. S1.- Soluble PD-L1 (sPD-L1) levels across clinical stages of decompensated cirrhosis among patients from Cohorts 1 and 2. Boxplots represent the interquartile range (IQR) with horizontal lines indicating the 25th and 75th percentiles. Individual dots represent patient-level sPD-L1 values. Pairwise comparisons were performed using Wilcoxon rank-sum tests.

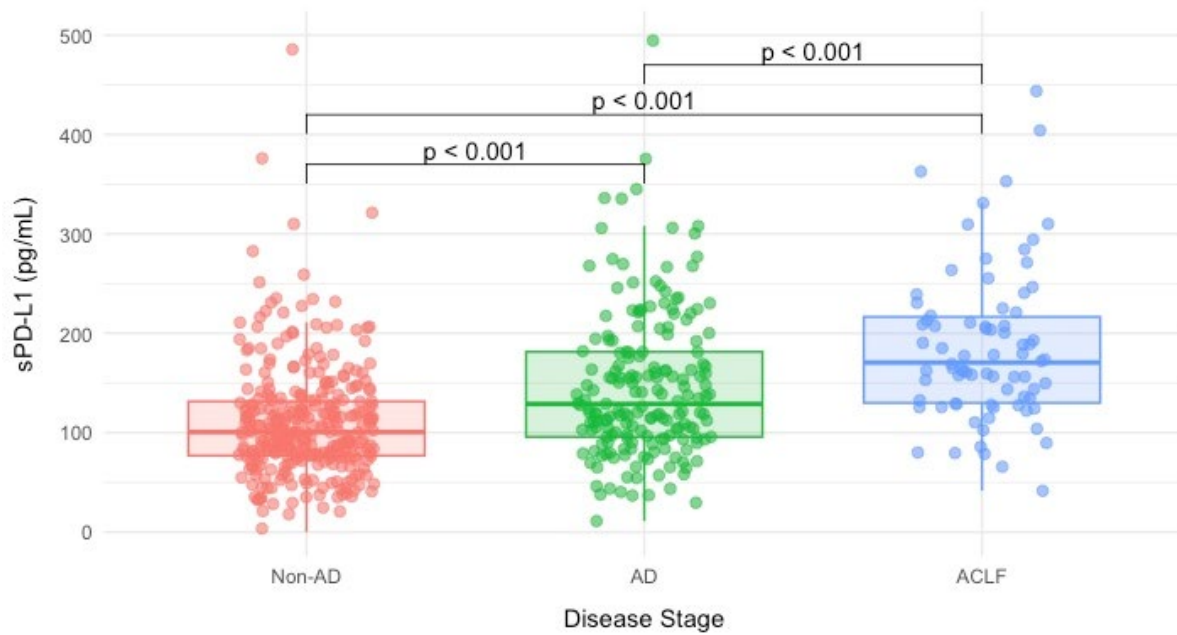


Fig. S2.- Cumulative incidence curves of bacterial infections in patients from cohort 2 categorized according to sPD-L1 median levels. The blue curve represents patients with sPD-L1 < 147 pg/mL, and the red curve represents those with sPD-L1 ≥ 147 pg/mL. The log-rank test was used to compare survival distributions between groups, with a statistically significant difference observed (p < 0.001). Number at risk is displayed below the time axis.

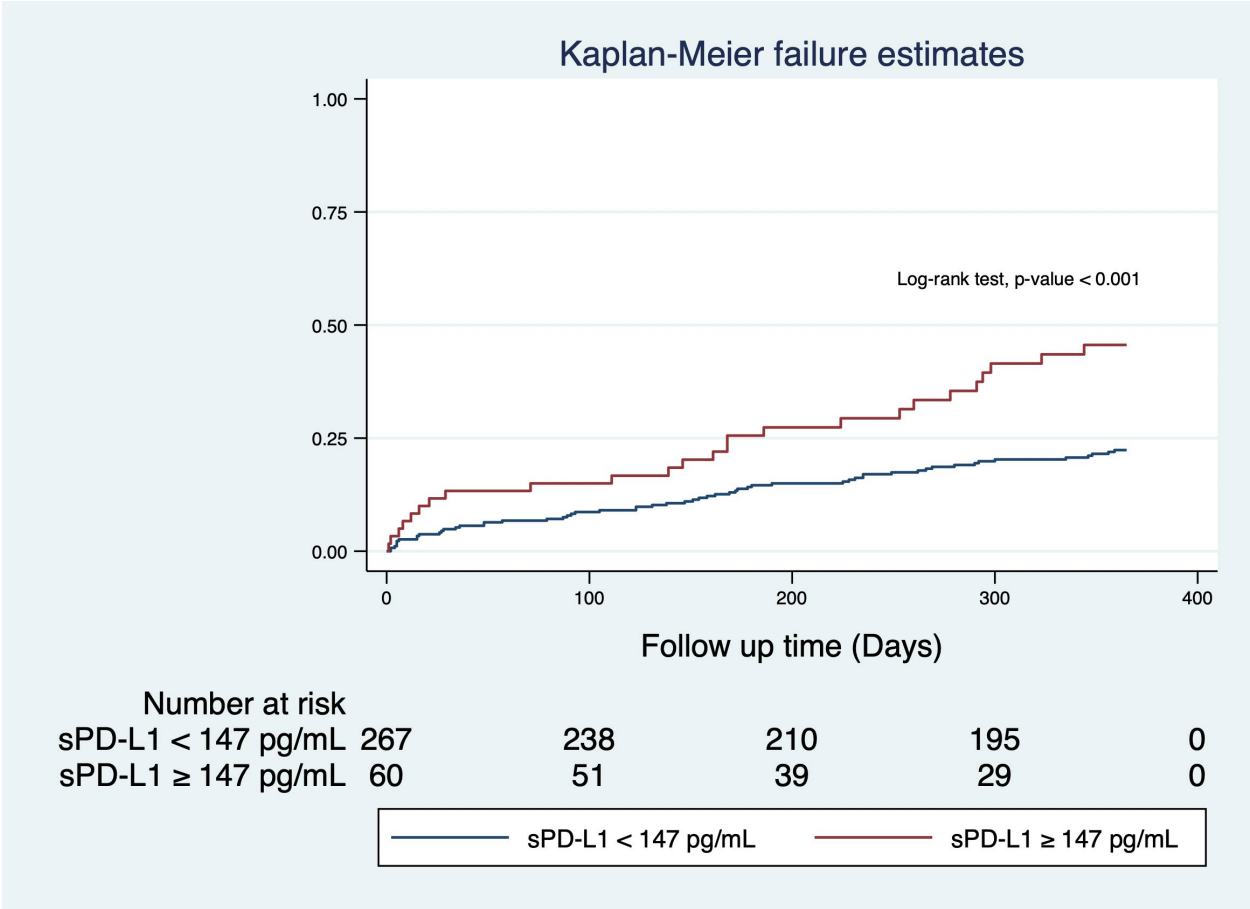


Fig. S3.- Soluble PD-L1 adjusted HR with potential confounders identified in the univariate analysis for bacterial infection development in patients from Cohort 3. Each diamond represents the adjusted hazard ratio (HR) of sPD-L1 when accounting for the indicated variable, based on a Cox proportional hazards model. The blue horizontal lines indicate the 95%CI for each adjusted HR. The grey dashed vertical line marks the unadjusted HR of sPD-L1, and the grey dotted lines represent its corresponding 95%CI. Variables included in this analysis were selected based on significance in the univariate analysis.

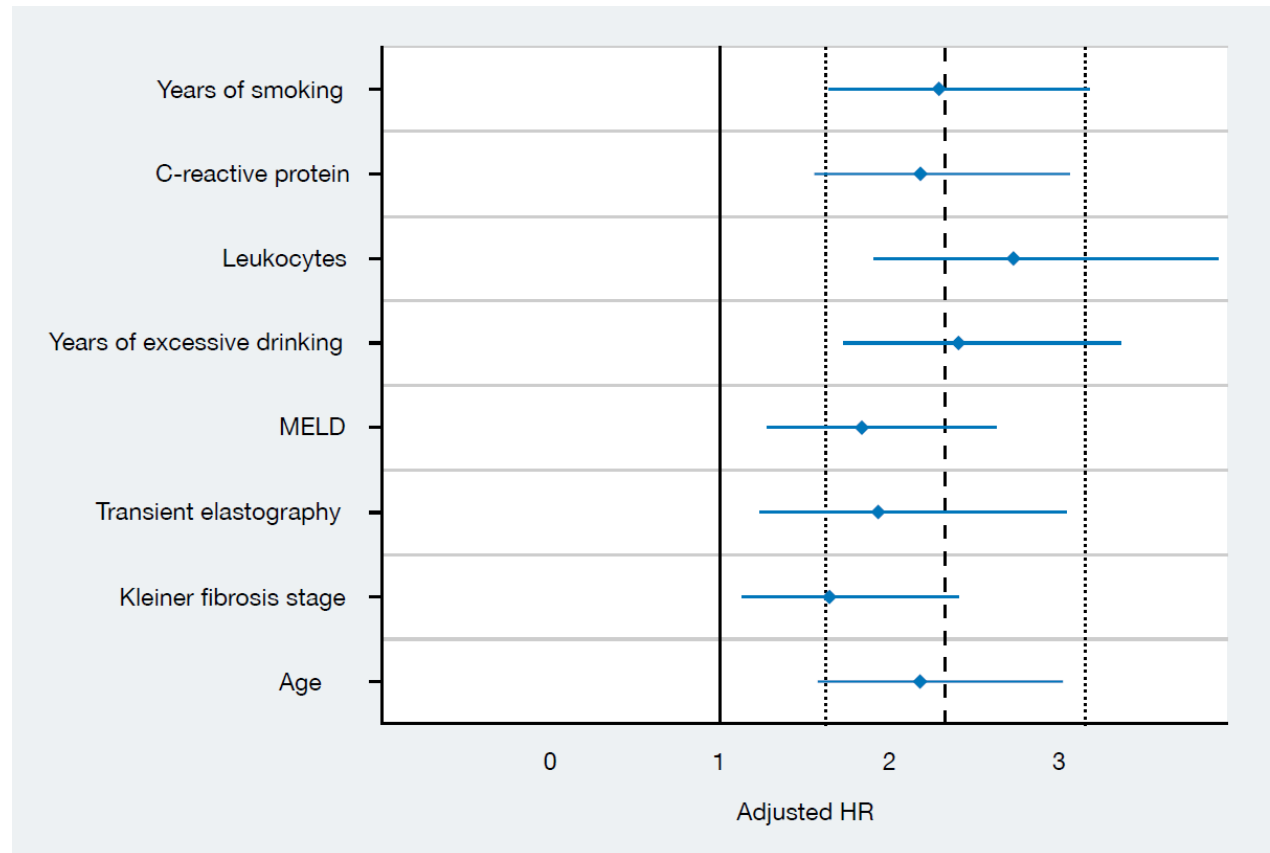


Fig. S4.- Cumulative incidence curves of bacterial infections in patients from cohort 3 categorized according to sPD-L1 median level. The blue curve represents patients with sPD-L1 < 5.7, and the red curve represents those with sPD-L1 ≥ 5.7. The log-rank test was used to compare survival distributions between groups, with a statistically significant difference observed ($p < 0.001$). Number at risk is displayed below the time axis.

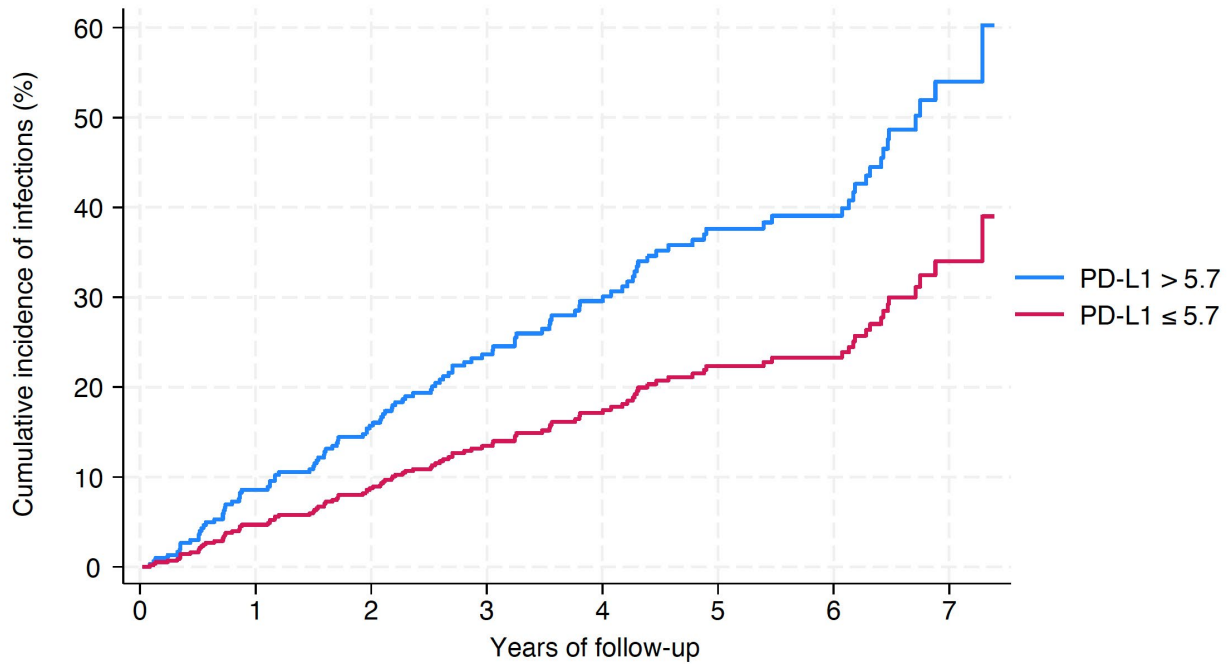


Fig. S5.- Soluble PD-L1 adjusted HR with potential confounders identified in the univariate analysis for 90-day mortality in patients from Cohort 1. Each diamond represents the adjusted hazard ratio (HR) of sPD-L1 when accounting for the indicated variable, based on a Cox proportional hazards model. The blue horizontal lines indicate the 95% CIs for each adjusted HR. The grey dashed vertical line marks the unadjusted HR of sPD-L1, and the grey dotted lines represent its corresponding 95% CI. Variables included in this analysis were selected based on significance in the univariate analysis.

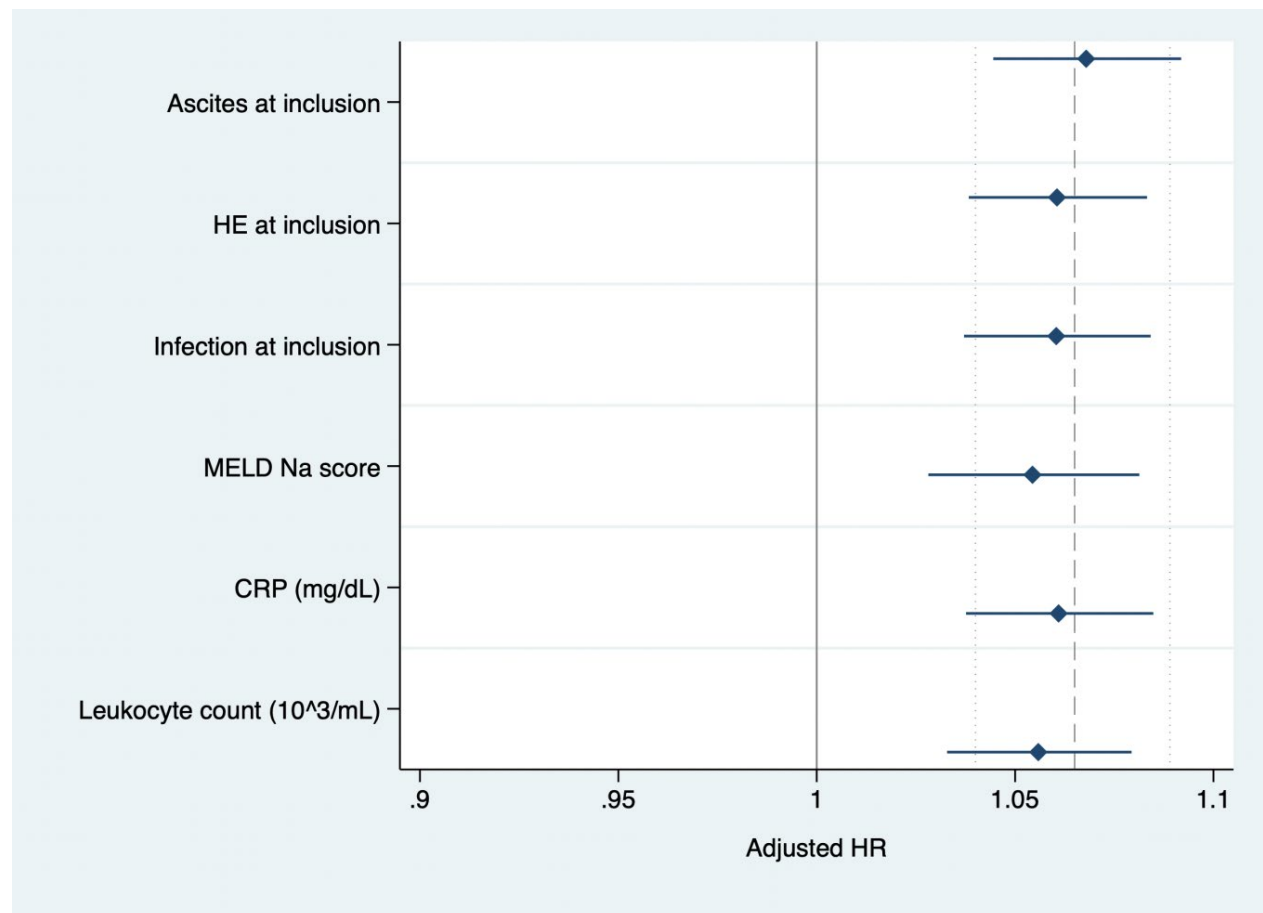


Fig. S6.- Soluble PD-L1 adjusted HR with potential confounders identified in the univariate analysis for 1-year mortality in patients from Cohort 2. Each diamond represents the adjusted hazard ratio (HR) of sPD-L1 when accounting for the indicated variable, based on a Cox proportional hazards model. The blue horizontal lines indicate the 95%CI for each adjusted HR. The grey dashed vertical line marks the unadjusted HR of sPD-L1, and the grey dotted lines represent its corresponding 95%CI. Variables included in this analysis were selected based on significance in the univariate analysis.

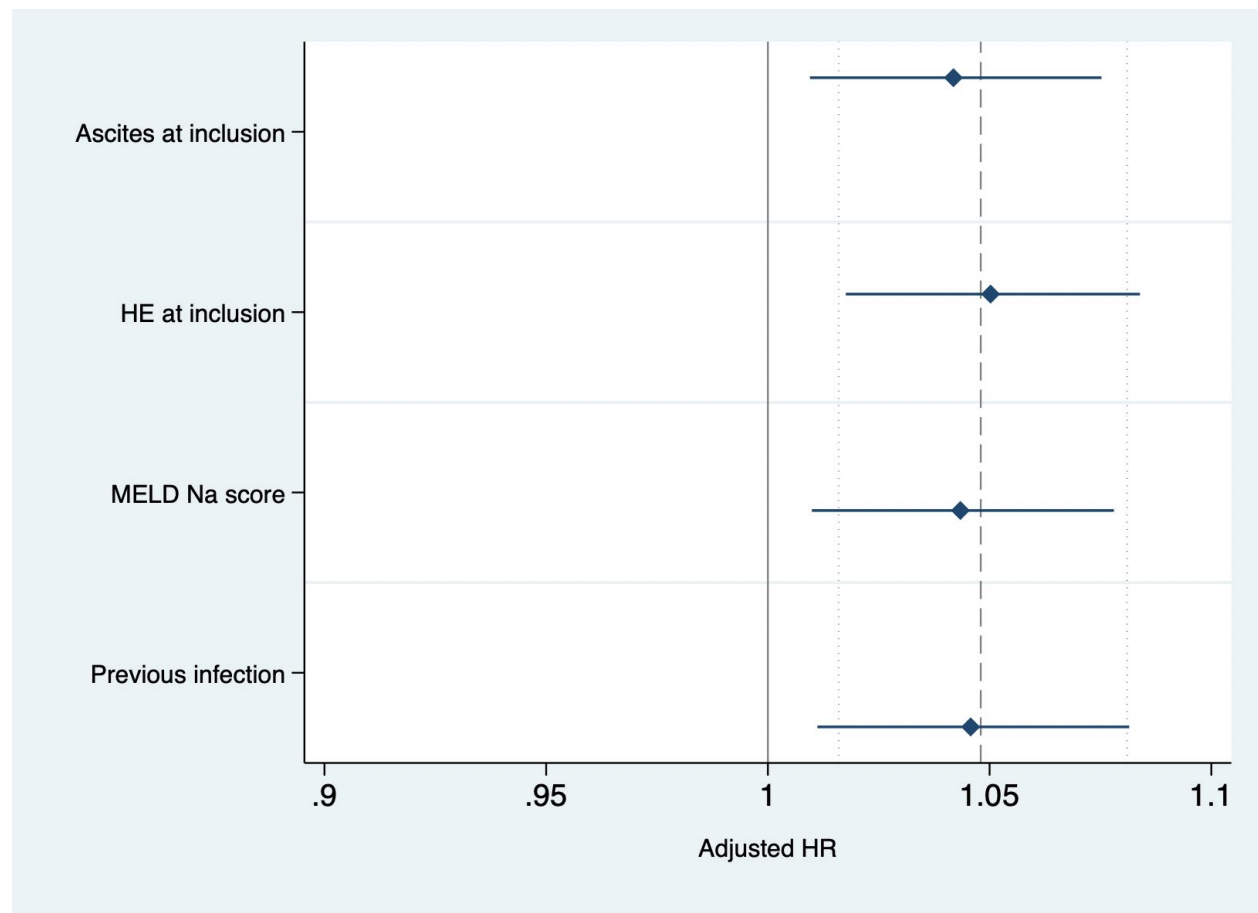


Fig. S7.- Kaplan–Meier survival curves in patients from Cohort 2 categorized by median sPD-L1 plasma levels. Kaplan–Meier survival estimates for 1-year mortality in patients from Cohort 2 stratified by soluble PD-L1 (sPD-L1) plasma levels using the cut-off value of 147 pg/mL. The blue curve represents patients with sPD-L1 < 147 pg/mL, and the red curve represents those with sPD-L1 ≥ 147 pg/mL. The log-rank test was used to compare survival distributions between groups, with a statistically significant difference observed (p = 0.0185). Number at risk is displayed below the time axis.

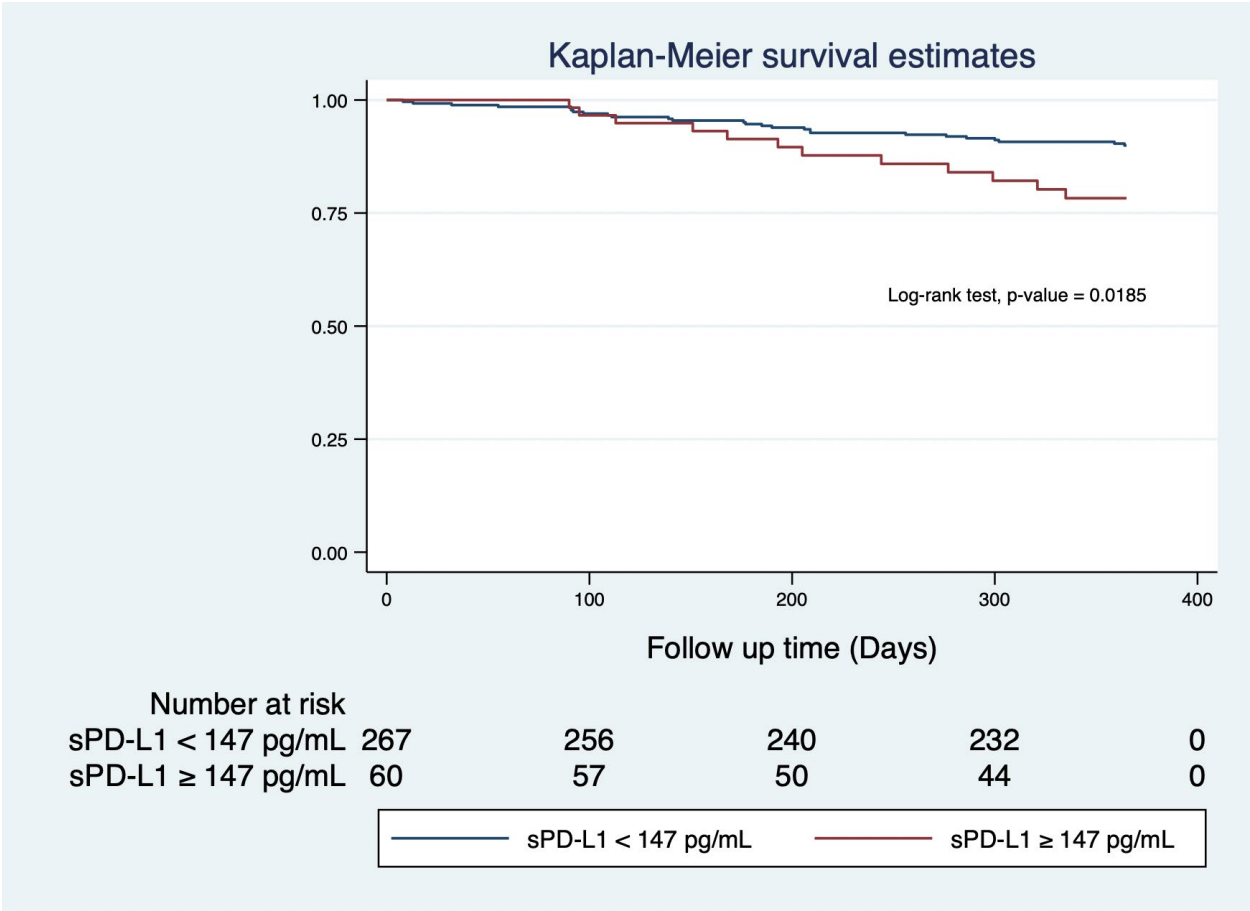


Fig. S8.- Soluble PD-L1 adjusted HR with potential confounders identified in the univariate analysis for mortality in patients from Cohort 3. Each diamond represents the adjusted hazard ratio (HR) of sPD-L1 when accounting for the indicated variable, based on a Cox proportional hazards model. The blue horizontal lines indicate the 95% CIs for each adjusted HR. The grey dashed vertical line marks the unadjusted HR of sPD-L1, and the grey dotted lines represent its corresponding 95%CI. Variables included in this analysis were selected based on significance in the univariate analysis.

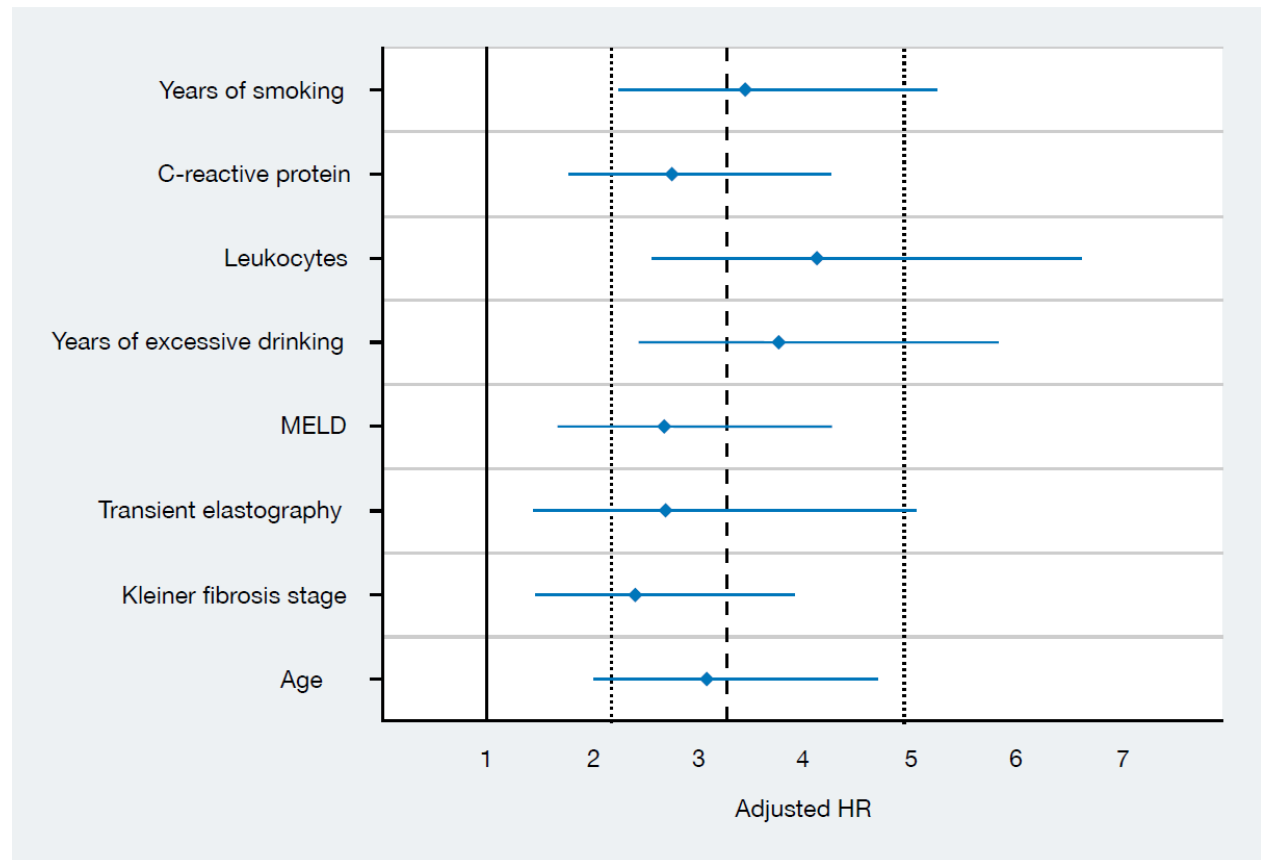
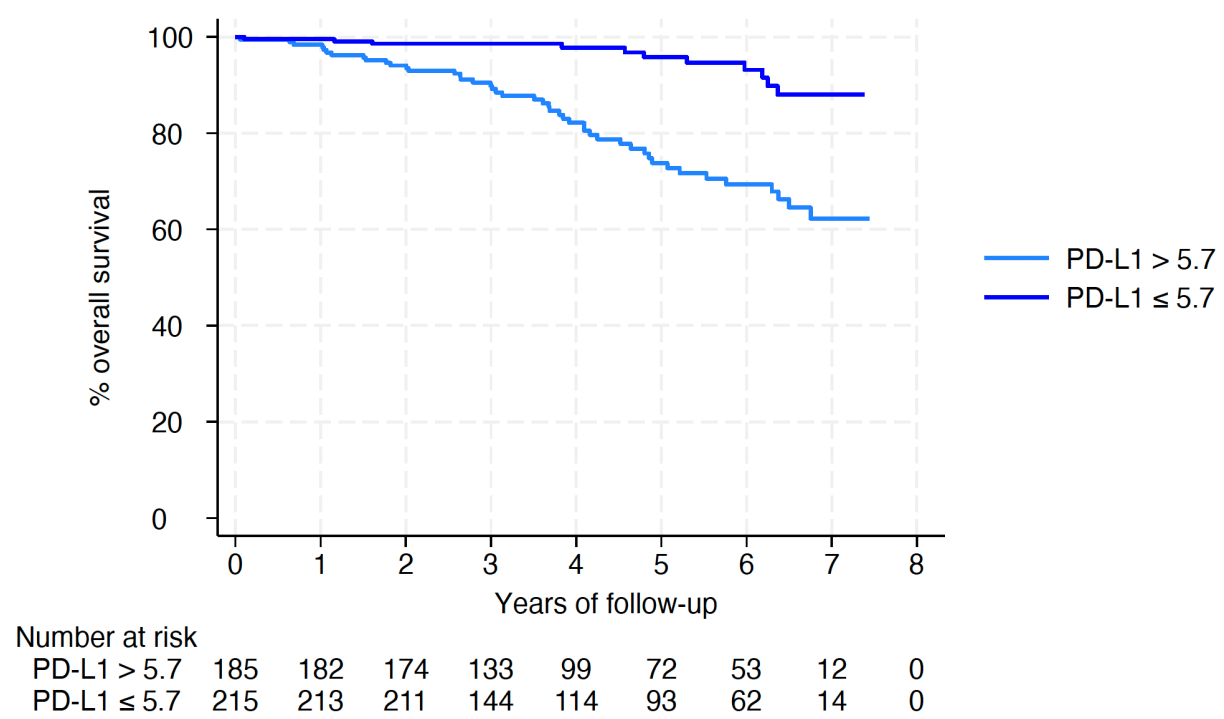


Fig. S9.- Kaplan Meier survival curves in patients from cohort 3 categorized according to sPD-L1 median levels. The dark blue curve represents patients with sPD-L1 < 5.7, and the light blue curve represents those with sPD-L1 ≥ 5.7. The log-rank test was used to compare survival distributions between groups, with a statistically significant difference observed (p < 0.001). Number at risk is displayed below the time axis.



Supplementary tables

Table S1 – Inclusion and Exclusion Criteria for the Different Cohorts.

Inclusion Criteria	Exclusion Criteria
Cohort 1 (n = 268)	
Patients with decompensated cirrhosis hospitalized for management of an acute decompensation episode; or, patients with liver cirrhosis visited at outpatient clinic.	<ul style="list-style-type: none"> (1) hemodialysis before admission (2) liver and/or kidney transplantation (3) admission for elective diagnostic or therapeutic procedures (4) advanced hepatocellular carcinoma beyond Milan criteria (5) severe extrahepatic diseases with poor short-term prognosis.
Cohort 2: Patients from the Liverhope Efficacy Trial (n = 214)	
Patients 18 years of age or older, with a clinical diagnosis of decompensated cirrhosis, namely Child-Pugh class B or C.	<ul style="list-style-type: none"> (1) presence of ACLF at enrollment (2) serum bilirubin >5 mg/dL (85.5 µmol/L) (3) INR ≥2.5 (4) severe alcohol-associated hepatitis requiring corticosteroid therapy (5) overt HE (6) Child-Pugh score ≥ 13. (7) severe extrahepatic comorbidities, including congestive heart failure New York Heart Association Grade III/IV, chronic obstructive pulmonary disease Global Initiative for Chronic Obstructive Lung Disease group 2 or higher (8) serum creatinine >2mg/dL (176.8 µmol/L) or on renal replacement therapy (9) patients on treatment with rifaximin or statins (10) increased risk of adverse events related to the study medications (i.e treatment with potent inhibitors of CYP3A4) or hypersensitivity to study drugs were excluded. (11) patients unable to provide consent or with anticipated poor compliance (12) alcohol consumption ≥3 units per day were also excluded.

Cohort 2: Patients from Padova Cohort (n = 113)	
<p>(1) Age >18 years;</p> <p>(2) Non-acute decompensated cirrhosis diagnosed by histological findings on biopsy, or by the evidence of clinical, biohumoral or instrumental data (endoscopic, ultrasound or liver stiffness measured by transient elastography);</p> <p>(3) Ability and will to provide written informed consent.</p>	<p>(1) diagnosis of hepatocellular carcinoma (HCC) or extrahepatic malignancies at the time of inclusion;</p> <p>(2) other highly disabling pathologies of extrahepatic origin at the time of inclusion (e.g. heart failure [NYHA class ≥ 3] or GOLD chronic obstructive pulmonary disease grade ≥ 3);</p> <p>(3) recurrence of cirrhosis after liver transplantation (LT);</p> <p>(4) refusal, or inability of the patient to provide informed consent.</p>
Cohort 3: GALA-ALD cohort (n = 400)	
<p>(1) Age 18-75 years</p> <p>(2) Prior or current chronic alcohol overuse defined as >24 g/d for women and >36 g/d for men for >1 year</p> <p>(3) Informed consent</p>	<p>(1) Previous or current decompensation Concurrent liver disease other than ALD</p> <p>(2) Cancer or other debilitating disease with an expected survival of <12 months</p> <p>(3) Severe alcoholic hepatitis, hepatic congestion or bile duct dilation evidenced by ultrasound</p> <p>(4) Positive for human immunodeficiency virus</p> <p>(5) Ongoing substance abuse other than alcohol</p> <p>(6) Contraindication to a liver biopsy</p> <p>(7) Inability to comply with the study protocol</p>

Table S2 - Type distribution of the first bacterial infection during follow-up.

Type of First Infection	Cohort 1 (n = 268)	Cohort 2 (n = 327)	Cohort 3 (n = 400)
Urinary Tract Infection	36 (36)	15 (27)	16 (15)
Respiratory tract infection	21 (21)	5 (9)	41 (38)
Spontaneous bacteremia	11 (11)	5 (9)	8 (7)
Spontaneous bacterial peritonitis	9 (9)	12 (22)	2 (2)
Skin & Soft tissue infection	9 (9)	8 (15)	13 (12)
Other	15 (14)	10 (18)	28 (26)

Table S3.- Univariate analysis for factors associated with 3-month bacterial infection development in patients from Cohort 1. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were estimated using Cox proportional hazards models. P-values correspond to the Wald test for the significance of each covariate.

Variable	No Infection during follow-up (n = 167)	Infection during follow-up (n = 101)	HR (95%CI)	p value
Age	60 (53 - 67)	60 (52 - 65)	0.997 (0.980 - 1.015)	0.764
Sex	45 (27)	28 (28)	0.977 (0.632 - 1.510)	0.916
Etiology				
Alcohol	102 (61)	63 (62)	Ref.	Ref.
HVC	27 (16)	11 (11)	0.639 (0.337 - 1.213)	0.171
Alcohol + Viral	13 (8)	8 (8)	1.090 (0.522 - 2.274)	0.819
MASLD	10 (6)	12 (12)	1.489 (0.802 - 2.761)	0.207
Other	15 (9)	7 (7)	0.757 (0.347 - 1.654)	.0486
Ascites at inclusion	104 (62)	75 (74)	1.794 (1.147 - 2.804)	0.010
HE at inclusion	50 (30)	44 (44)	1.841 (1.240 - 2.734)	0.002
Previous infection	42 (25)	48 (48)	1.917 (1.296 - 2.833)	0.001
Infection at inclusion	86 (52)	59 (58)	1.338 (0.900 - 1.988)	0.150
Bilirubin (mg/dL)	2.4 (1.2 - 5.4)	3.2 (1.6 - 9.1)	1.045 (1.023 - 1.067)	<0.001
INR	1.46 (1.28 - 1.79)	1.70 (1.43 - 2.03)	1.168 (1.015 - 1.344)	0.030
Albumin (g/L)	29 (25 - 33)	28 (25 - 32)	0.986 (0.952 - 1.022)	0.450
Creatinine (mg/dL)	0.93 (0.60 - 1.45)	1.17 (0.70 - 2.02)	1.294 (1.135 - 1.475)	<0.001
Serum Na (mEq/L)	137 (133 - 140)	135 (132 - 138)	0.926 (0.893 - 0.959)	<0.001
Leukocyte count (10 ³ /mm ³)	5.3 (3.6 - 7.8)	5.9 (4.2 - 8.7)	1.055 (1.021 - 1.091)	0.001
CRP (mg/dL)	1.9 (0.8 - 4.9)	2.8 (1.2 - 5.1)	1.037 (0.994 - 1.081)	0.089
MELD Na score	19 (15 - 25)	24 (19 - 29)	1.090 (1.062 - 1.118)	<0.001
sPD-L1 (pg/mL)	136 (97 - 193)	159 (116 - 221)	1.034 (1.014 - 1.055)	0.001

Table S4.- Univariate analysis for factors associated with 1-year bacterial infection development in patients from Cohort 2. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were estimated using Cox proportional hazards models. P-values correspond to the Wald test for the significance of each covariate.

Variable	No Infection during follow-up (n = 245)	Infection during follow-up (n = 82)	HR (95%CI)	p value
Age	58 (52 - 64)	59 (53 - 68)	1.020 (0.998 - 1.042)	0.080
Sex	62 (25)	23 (28)	1.031 (0.637 - 1.670)	0.900
Etiology				
Alcohol	185 (75)	48 (59)	Ref.	Ref.
HVC	14 (6)	9 (11)	2.102 (1.031 - 4.284)	0.041
Alcohol +	6 (2)	2 (2)	1.282 (0.312 - 5.276)	0.731
Viral	14 (6)	12 (15)	2.430 (1.288 - 4.582)	0.006
MASLD	26 (11)	11 (13)	1.432 (0.743 - 2.759)	0.284
Other				
Ascites at inclusion	155 (63)	71 (87)	3.287 (1.741 - 6.206)	< 0.001
HE at inclusion	11 (4)	27 (33)	2.645 (1.363 - 5.133)	0.004
Previous infection	35 (14)	32 (39)	3.127 (2.005 - 4.878)	< 0.001
Bilirubin (mg/dL)	1.9 (1.2 - 2.8)	2.2 (1.4 - 3.4)	1.237 (1.102 - 1.388)	< 0.001
INR	1.31 (1.18 - 1.47)	1.34 (1.22 - 1.44)	0.879 (0.531 - 1.457)	0.618
Creatinine (mg/dL)	0.78 (0.66 - 0.94)	0.83 (0.66 - 1.05)	0.987 (0.847 - 1.151)	0.871
Serum Na (mEq/L)	138 (135 - 139)	136 (134 - 138)	0.937 (0.892 - 0.984)	0.010
Leukocyte count (10³/mm³)	5.3 (3.9 - 6.7)	4.8 (4.0 - 6.6)	0.971 (0.878 - 1.073)	0.562
MELD Na score	13 (9 - 16)	15 (10 - 18)	1.052 (1.015 - 1.089)	0.005
sPD-L1 (pg/mL)	92 (75 - 126)	116 (93 - 161)	1.042 (1.020 - 1.066)	< 0.001

Table S5 – Univariate analysis for factors associated to bacterial infection development in patients from Cohort 3. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were estimated using Cox proportional hazards models. P-values correspond to the Wald test for the significance of each covariate.

Variable	No Infection FU (N = 292)	Infection FU (N = 108)	HR (95%IC)	p value
Age	57 (50-63)	59 (52-66)	1.02 (1.00-1.04)	0.030
Kleiner fibrosis stage F0-1/F2/F3/F4	197/59/12/24	41/29/8/30	1.58 (1.36-1.85)	<0.001
LSM (kPa)	5.6 (4.4-9)	10.4 (5.7-28.4)	1.03 (1.02-1.03)	<0.001
Years of excessive drinking	16 (8-26)	16 (8-26)	1.00 (0.99-1.02)	0.981
Years of smoking	25 (8-37)	35 (13-45)	1.01 (1.00-1.02)	0.064
MELD score	6 (6-7)	7 (6-9)	1.24 (1.15-1.34)	<0.001
Leukocytes (10⁹/L)	6.6 (5.2-8.0)	7.0 (5.3-9.2)	1.04 (0.95-1.13)	0.377
CRP (mg/L)	2.2 (1.0-4.7)	3.1 (1.6-7.6)	1.00 (0.99-1.01)	0.758
PD-L1 (NPX)	5.6 (5.3-5.9)	5.9 (5.6-6.3)	2.30 (1.67-3.16)	<0.001

Table S6 - Univariate analysis for factors associated to 3-month mortality in patients from Cohort

1. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were estimated using Cox proportional hazards models. P-values correspond to the Wald test for the significance of each covariate.

Variable	Alive (N = 208)	Dead (N = 60)	HR (95%IC)	p value
Age	59 (53 - 67)	61 (53 - 67)	1.003 (0.980 - 1.026)	0.815
Sex	57 (27)	16 (27)	0.960 (0.542 - 1.701)	0.889
Etiology				
Alcohol	131 (63)	34 (57)	Ref.	Ref.
HVC	29 (14)	9 (15)	1.100 (0.528 - 2.294)	0.798
Alcohol + Viral	12 (6)	9 (15)	2.325 (1.114 - 4.850)	0.025
MASLD	18 (8.5)	4 (6.5)	0.848 (0.301 - 2.389)	0.755
Other	18 (8.5)	4 (6.5)	0.957 (0.340 - 2.698)	0.934
Ascites at inclusion	128 (62)	51 (85)	3.218 (1.584 - 6.539)	0.001
HE at inclusion	60 (29)	34 (57)	2.940 (1.763 - 4.903)	<0.001
Previous infection	71 (34)	19 (32)	0.851 (0.494 - 1.466)	0.561
Infection at inclusion	104 (50)	41 (68)	1.984 (1.151 - 3.419)	0.014
Bilirubin (mg/dL)	2.1 (1.2 - 4.4)	8.7 (3.3 - 16.3)	1.073 (1.051 - 1.095)	<0.001
INR	1.46 (1.28 - 1.76)	2.00 (1.57 - 2.34)	1.318 (1.167 - 1.488)	<0.001
Creatinine (mg/dL)	0.92 (.060 - 1.44)	1.62 (0.99 - 2.26)	1.404 (1.206 - 1.636)	<0.001
Serum Na (mEq/L)	136 (134 - 140)	134 (127 - 137)	0.885 (0.848 - 0.924)	<0.001
Leukocyte count (10³/mm³)	5.0 (3.6 - 7.3)	7.9 (5.3 - 12.6)	1.104 (1.069 - 1.139)	<0.001
CRP (mg/dL)	1.9 (0.8 - 4.5)	3.1 (1.3 - 5.6)	1.060 (1.009 - 1.114)	0.021
MELD Na score	20 (15 - 24)	30 (24 - 34)	1.162 (1.126 - 1.198)	<0.001
sPD-L1 (pg/mL)	134 (97 - 187)	180 (143 - 267)	1.066 (1.043 - 1.089)	<0.001

Table S7 - Multivariate analysis for factors associated with 90-day mortality in patients from Cohort 1. Stepwise forward Cox analysis including factors with positive association (p value < 0.05) in the univariate analysis.

Variable	p value	HR	95%CI
sPD-L1 (pg/mL)	< 0.001	1.049 [#]	1.023 - 1.077 [#]
MELD sodium score	< 0.001	1.122	1.090 - 1.155
Leukocyte count (x10 ³ /mm ³)	0.004	1.053	1.016 - 1.091
HE at inclusion	0.030	1.816	1.061 - 3.110

[#]HR evaluated per 10 units

CRP, C-reactive protein; HE, hepatic encephalopathy; HR, hazard ratio; MELD, model for end-stage liver disease; sPD-L1, soluble programmed death-ligand 1.

Table S8 - Univariate analysis for factors associated to 1-year mortality in patients from Cohort 2. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were estimated using Cox proportional hazards models. P-values correspond to the Wald test for the significance of each covariate.

Variable	Alive (N = 289)	Dead (N = 38)	HR (95%CI)	p value
Age	58 (52 - 64)	62 (54 - 69)	1.032 (0.999 - 1.065)	0.056
Sex	77 (27)	8 (21)	0.718 (0.329 - 1.566)	0.405
Etiology				
Alcohol	206 (71)	27 (71)	Ref.	Ref.
HVC	18 (6)	5 (13)	1.989 (0.766 - 5.165)	0.158
Alcohol + Viral	8 (3)	0 (0)	NA	1.000
MASLD	22 (8)	4 (11)	1.401 (0.490 - 4.003)	0.529
Other	35 (12)	2 (5)	0.471 (0.112 - 1.981)	0.304
Ascites at inclusion	193 (67)	33 (87)	3.104 (1.212 - 7.952)	0.018
HE at inclusion	15 (5)	6 (16)	3.306 (1.381 - 7.911)	0.007
Previous infection	54 (19)	13 (34)	2.143 (1.096 - 4.190)	0.026
Bilirubin (mg/dL)	1.9 (1.2 - 2.8)	2.7 (1.6 - 3.5)	1.242 (1.106 - 1.394)	<0.001

INR	1.31 (1.18 - 1.46)	1.36 (1.24 - 1.51)	1.014 (0.732 - 1.405)	0.932
Creatinine (mg/dL)	0.78 (0.67 - 1.00)	0.82 (0.69 - 0.98)	1.070 (1.036 - 1.104)	<0.001
Serum Na (mEq/L)	137 (135 - 139)	136 (134 - 138)	0.934 (0.871 - 1.002)	0.056
Leukocyte count (10³/mm³)	5.2 (4.0 - 6.7)	4.9 (4.0 - 6.0)	0.928 (0.794 - 1.084)	0.345
MELD Na score	13 (9 - 16)	16 (10 - 20)	1.072 (1.021 - 1.125)	0.005
sPD-L1 (pg/mL)	99 (77 - 128)	121 (91 - 152)	1.048 (1.016 - 1.081)	0.003

Table S9 - Multivariate analysis for factors associated with 1-year mortality in patients from Cohort 2. Stepwise forward Cox analysis including HE, ascites, MELD Na score, leucocyte count and sPD-L1, according to its statistical significance ($p < 0.05$) in univariate analysis.

Variable	p value	HR	95%CI
sPD-L1 (pg/mL)	0.009	1.046 [#]	1.011 - 1.082 [#]
MELD sodium score	0.027	1.062	1.007 - 1.120
HE at inclusion	0.004	3.665	1.523 - 8.824

[#] HR evaluated per 10 units

HE, hepatic encephalopathy; HR, hazard ratio; MELD, model for end-stage liver disease; sPD-L1, soluble programmed death-ligand 1.

Table S10 - Univariate analysis for factors associated to mortality in patients from Cohort 3. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were estimated using Cox proportional hazards models. P-values correspond to the Wald test for the significance of each covariate.

Variable	Alive (N = 344)	Dead (N = 56)	HR (95%CI)	p value
Age	57 (50-64)	60 (53-67)	1.03 (1.00-1.06)	0.036
Kleiner fibrosis stage F0-1/F2/F3/F4	223/71/16/34	15/17/4/20	1.72 (1.40-2.11)	<0.001
LSM (kPa)	5.8 (4.6-10)	15.9 (6.1-31.4)	1.02 (1.01-1.04)	<0.001
Years of excessive drinking	16 (8-26)	16 (8-26)	1.00 (0.98-1.03)	0.766
Years of smoking	25 (8-40)	35 (25-40)	1.02 (1.00-1.04)	0.026
MELD score	6 (6-7)	7 (6-9)	1.28 (1.16-1.41)	<0.001
Leukocytes (10⁹/L)	6.6 (5.3-8.3)	7.1 (5.4-8.4)	0.99 (0.88-1.11)	0.858
C-reactive protein (mg/L)	2.2 (1.0-4.8)	3.7 (2.3-6.7)	1.00 (0.99-1.02)	0.854
PD-L1 (NPX)	5.6 (5.3-5.9)	6.1 (5.7-6.5)	3.29 (2.19-4.95)	<0.001

Table S11 - Multivariate analysis for factors associated with mortality in patients from Cohort 3.

Variable	p-value	HR	95%CI
LSM (kPa)	0.022	1.01	1.00 - 1.03
sPD-L1	0.002	2.69	1.44 - 5.05

Stepwise forward Cox analysis, according to its statistical significance ($p < 0.05$) in univariate analysis. The total cohort includes 400 patients with 56 events. In this regression, 387 patients are included in a complete case analysis due to missing values of transient elastography ($n = 13$) and MELD ($n = 1$). HR, hazard ratio; LSM, liver stiffness measurement; MELD, model for end-stage liver disease; sPD-L1, soluble programmed death-ligand 1; TE, transient elastography.

Table S12.- STROBE Statement. Checklist of items that should be included in reports of cohort studies.

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	1 9
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	10,11
Objectives	3	State specific objectives, including any prespecified hypotheses	11
Methods			
Study design	4	Present key elements of study design early in the paper	12
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	12,13
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (b) For matched studies, give matching criteria and number of exposed and unexposed	12,13, S1
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	13-15
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	13-17
Bias	9	Describe any efforts to address potential sources of bias	16,17
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	16
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, explain how loss to follow-up was addressed (e) Describe any sensitivity analyses	16,17 16,17 NA 16 16,17
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	NA NA NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	18,19, T1 NA 12,13,19
Outcome data	15*	Report numbers of outcome events or summary measures over time	18-21

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	18-21, T2,T3,T4, F1,F2,F3,F4 18 NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	21
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	24
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	21-25
Generalisability	21	Discuss the generalisability (external validity) of the study results	21-25
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	4