Dysregulated biomarkers of innate and adaptive immunity predict infections and disease progression in cirrhosis

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Supplementary methods

Definition of liver-related events

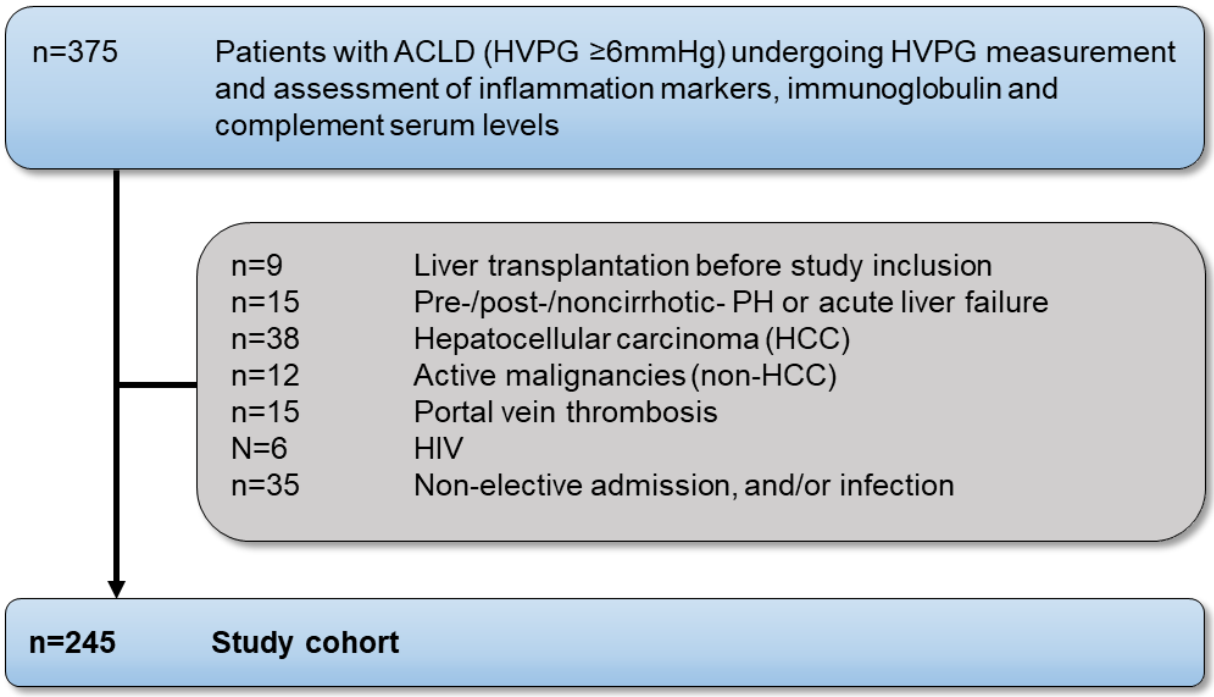
The composite endpoint “first/further decompensation or liver-related death” was defined as the incidence and/or worsening of ascites, variceal bleeding, hepatic encephalopathy (HE), or liver-related death (LRD) during follow-up. The timespan between hepatic venous pressure gradient (HVPG) measurement - denoting the baseline - and the closest follow-up event (FUE) was determined for statistical analyses assessing the prognostic value of variables. Patients were censored at the date of last clinical contact, of liver transplantation, or at 24 months after HVPG measurement if no FUE occurred. The timepoint of the third large-volume paracentesis within 6 months was defined as worsening of ascites in patients without refractory ascites who had already received treatment for ascites at the timepoint of HVPG measurement. The timepoint of admission for overt HE was defined as worsening of HE in patients who had already received treatment for mild HE but had no record of overt HE prior to HVPG measurement. Variceal bleeding was considered an event of further decompensation if occuring in patients with a previous history of variceal bleeding or in patients with other decompensation events.

Incidence of hepatocellular carcinoma

Four (1.6%) patients were diagnosed with hepatocellular carcinoma (HCC) during the follow-up period (n=1 with compensated, n=3 with decompensated ACLD at baseline). As by local standard operating procedures, HCC surveillance is performed by abdominal imaging (using ultrasound, computed tomography, or magnetic resonance imaging) and laboratory assessment of AFP levels every 6 months. Diagnosis of HCC was performed based on EASL clinical practice guidelines [1]. Due to the low number of events, no specific analyses on the prediction of HCC were performed.

Supplementary figures

Fig. S1. Study flow chart.



Abbreviations: (ACLD) advanced chronic liver disease; (HVPG) hepatic venous pressure gradient; (PH) portal hypertension; (HIV) Human immunodeficiency virus

Fig. S2. Complement levels and complement activity in patients stratified by portal hypertension severity and hepatic dysfunction.

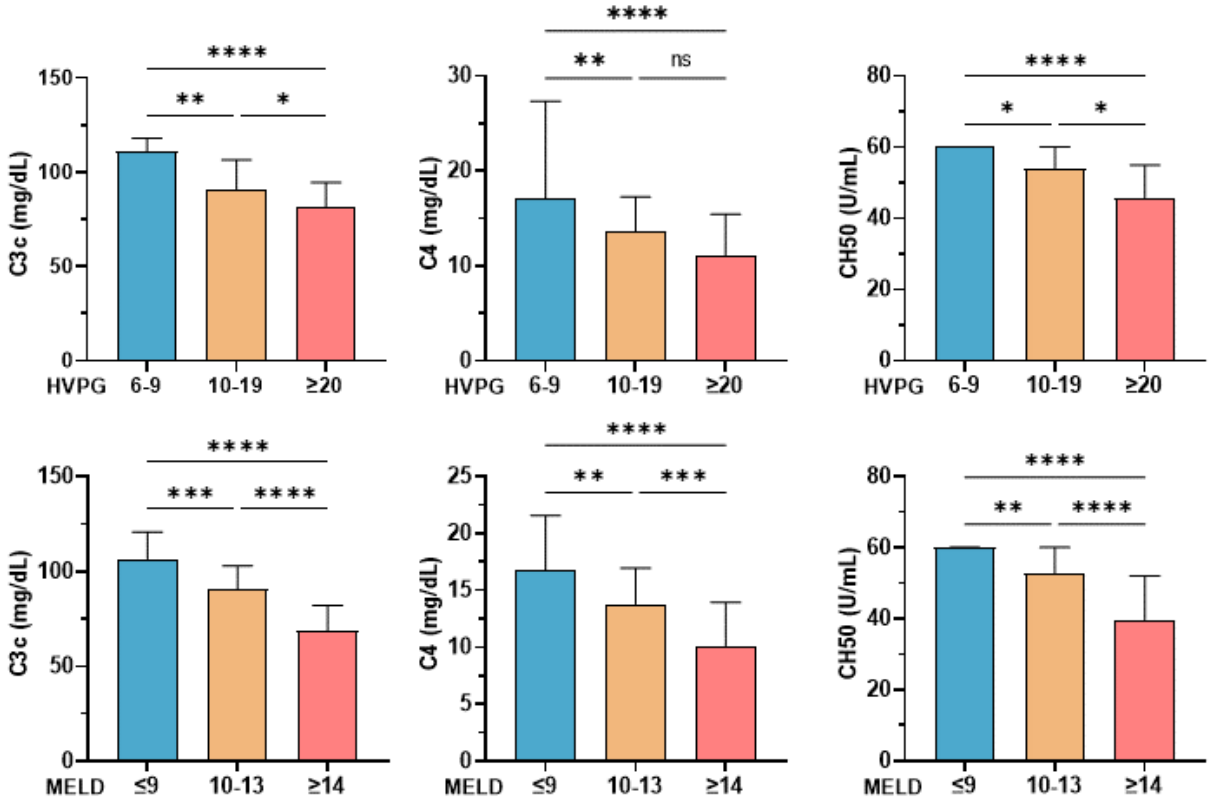


Figure legend: (ns) not significant; (*) p<0.05; (**) p<0.01; (***) p<0.001; (****) p<0.0001. Number of patients in HVPG subgroups: n=32 with 6-9, n=129 with 10-19, n=84 with ≥20 mmHg. Number of patients in MELD subgroups: n=80 with ≤9, n=85 with 10-13, n=80 with ≥14. Statistical analysis: Group comparisons were performed by Kruskal-Wallis test. Dunn's multiple comparisons test was applied. Abbreviations: (C3c) complement C3 component; (HVPG) hepatic venous pressure gradient; (MELD) Model of End Stage Liver Disease

Fig. S3. Immunoglobulin levels in patients with compensated and decompensated advanced chronic liver disease (ACLD).

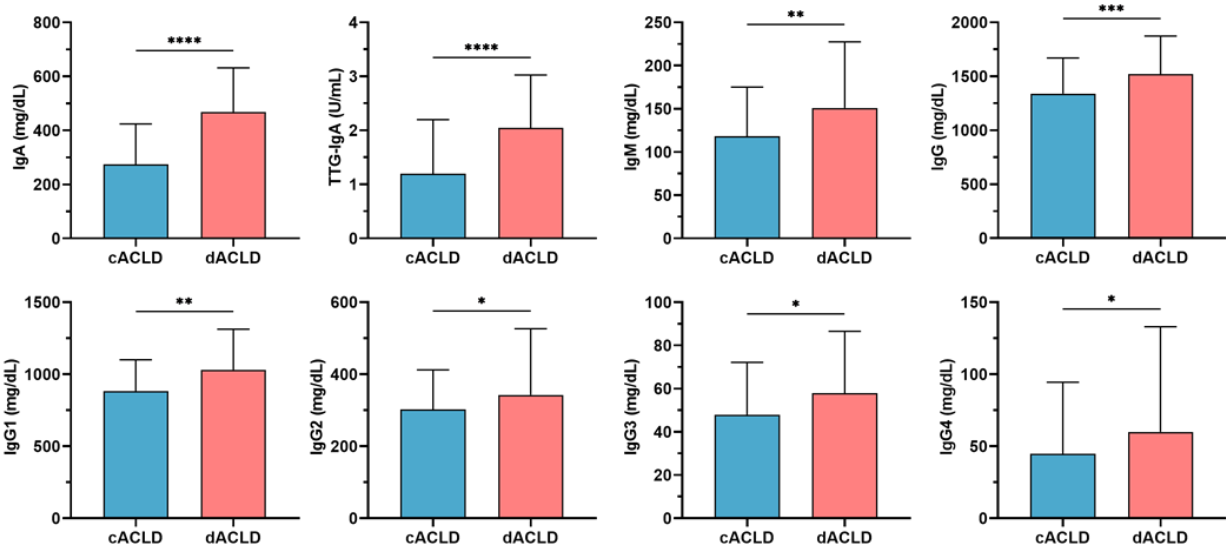


Figure legend: (ns) not significant; (*) p<0.05; (**) p<0.01; (***) p<0.001; (****) p<0.0001. Statistical analysis: Group comparisons were performed by Mann Whitney U test. Abbreviations: (Ig) immunoglobulin; (TTG) tissue transglutaminase; (c/dACLD) compensated/decompensated advanced chronic liver disease

Fig. S4. Immunoglobulin levels in patients stratified by portal hypertension severity and hepatic dysfunction.

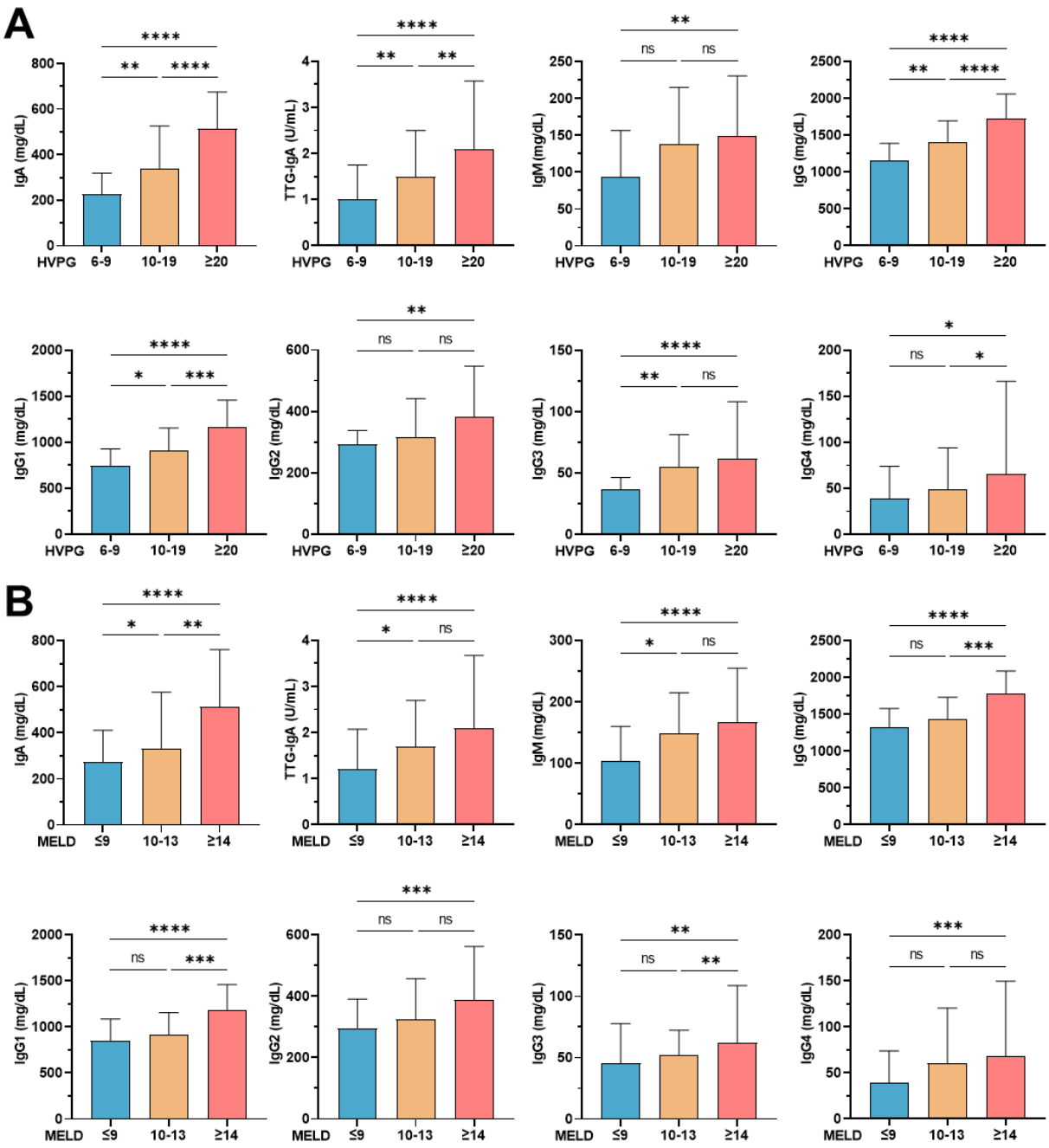
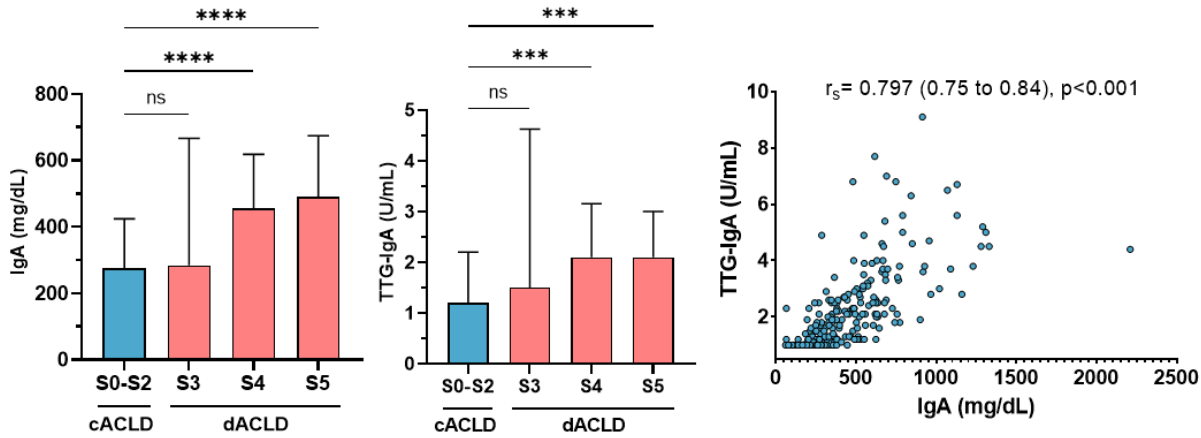


Figure legend: (ns) not significant; (*) p<0.05; (**) p<0.01; (***) p<0.001; (****) p<0.0001. Number of patients in HVPG subgroups: n=32 with 6-9, n=129 with 10-19, n=84 with ≥20 mmHg. Number of patients in MELD subgroups: n=80 with ≤9, n=85 with 10-13, n=80 with ≥14. Statistical analysis: Group comparisons were performed by Kruskal-Wallis test. Dunn's multiple comparisons test was applied. Abbreviations: (HVPG) hepatic venous pressure gradient; (MELD) Model of End Stage Liver Disease; (Ig) immunoglobulin; (TTG) tissue transglutaminase

Fig. S5. TTG-IgA across ACLD stages and correlation between IgA and TTG-IgA levels.



Statistical analysis: Spearman's correlation coefficient was calculated to assess the association between continous variables.

Abbreviations: (Ig) immunoglobulin; (TTG) tissue transglutaminase; (c/dACLD) compensated/decompensated advanced chronic liver disease

Fig. S6. IgG 1-4 levels across different stages of advanced chronic liver disease (ACLD).

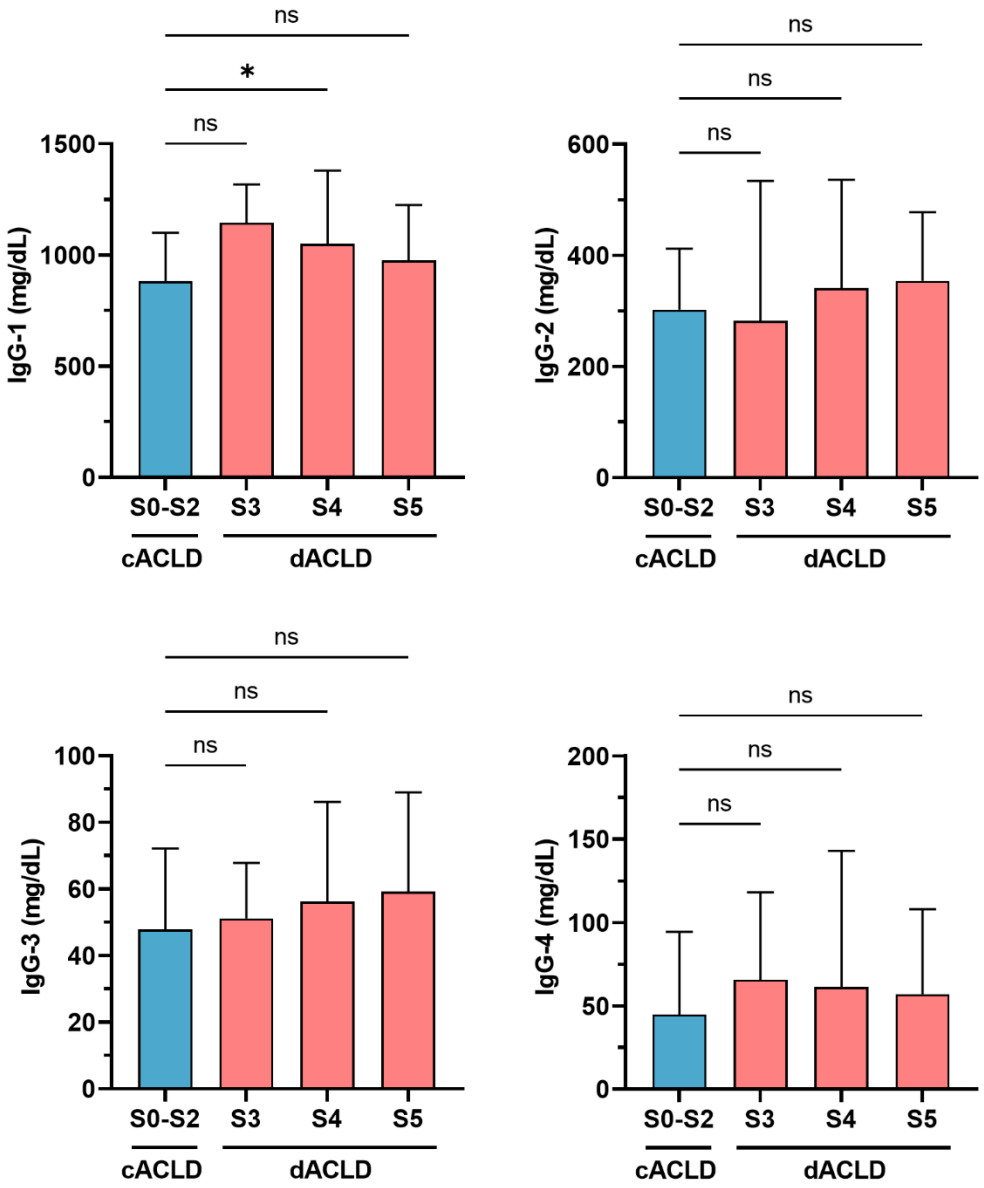


Figure legend: (ns) not significant; (*) p<0.05; (**) p<0.01; (***) p<0.001; (****) p<0.0001. Statistical analysis: Group comparisons were performed by Kruskal-Wallis test. Dunn's multiple comparisons test was applied. Abbreviations: (Ig) immunoglobulin; (c/dACLD) compensated/decompensated advanced chronic liver disease

Fig. S7. Acute-phase proteins across different stages of advanced chronic liver disease (ACLD).

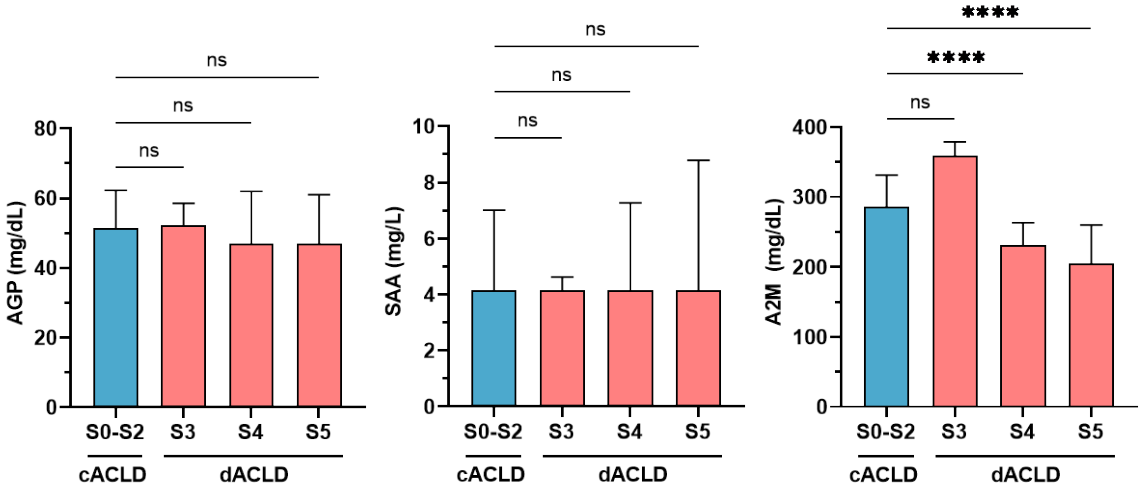
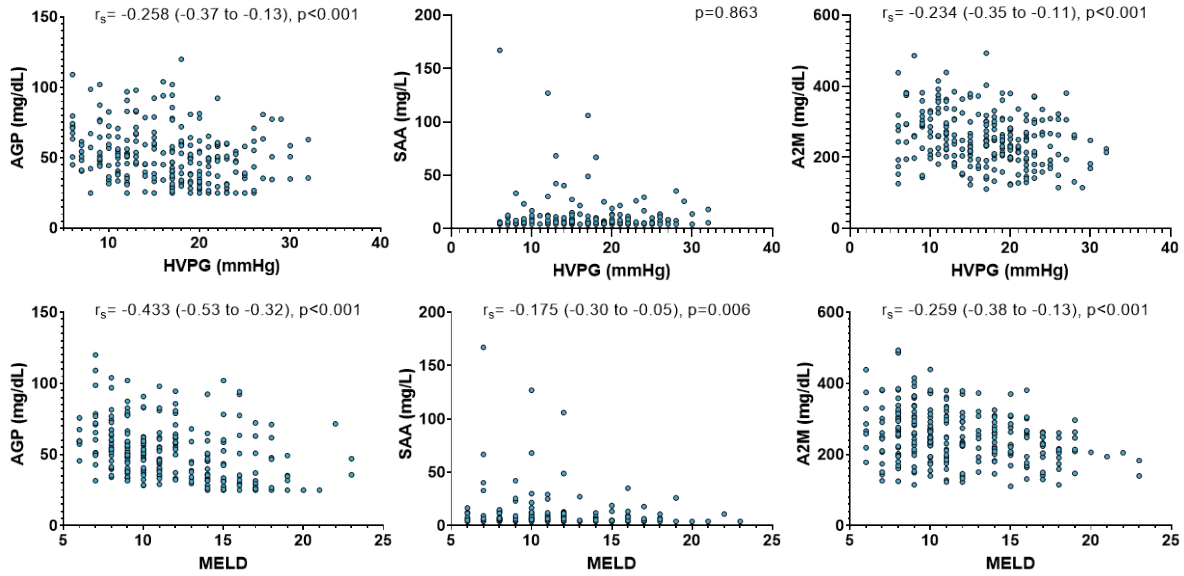


Figure legend: (ns) not significant; (*) p<0.05; (**) p<0.01; (***) p<0.001; (****) p<0.0001. Statistical analysis: Group comparisons were performed by Kruskal-Wallis test. Dunn's multiple comparisons test was applied.

Abbreviations: (c/dACLD) compensated/decompensated advanced chronic liver disease; (A2M) alpha-2-macroglobulin; (AGP) alpha-1-acid glycoprotein; (SAA) serum amyloid A

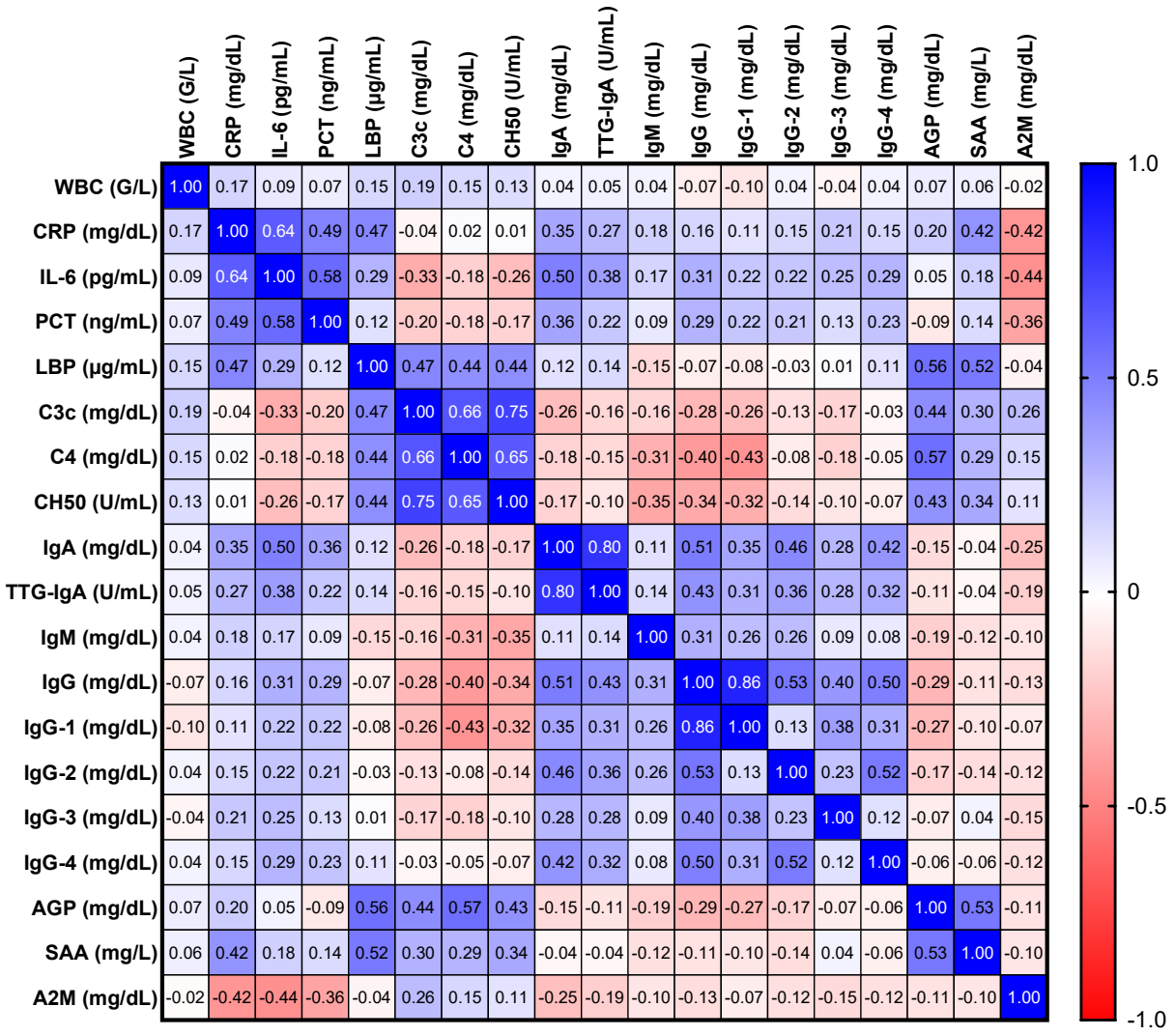
Fig. S8. Correlation between acute-phase proteins and severity of portal hypertension and hepatic dysfunction.



Statistical analysis: Spearman's correlation coefficient was calculated to assess the association between continous variables.

Abbreviations: (HVPG) hepatic venous pressure gradient; (MELD) Model of End Stage Liver Disease; (A2M) alpha-2-macroglobulin; (AGP) alpha-1-acid glycoprotein; (SAA) serum amyloid A

Fig. S9. Correlation matrix of immunological parameters in the systemic circulation.

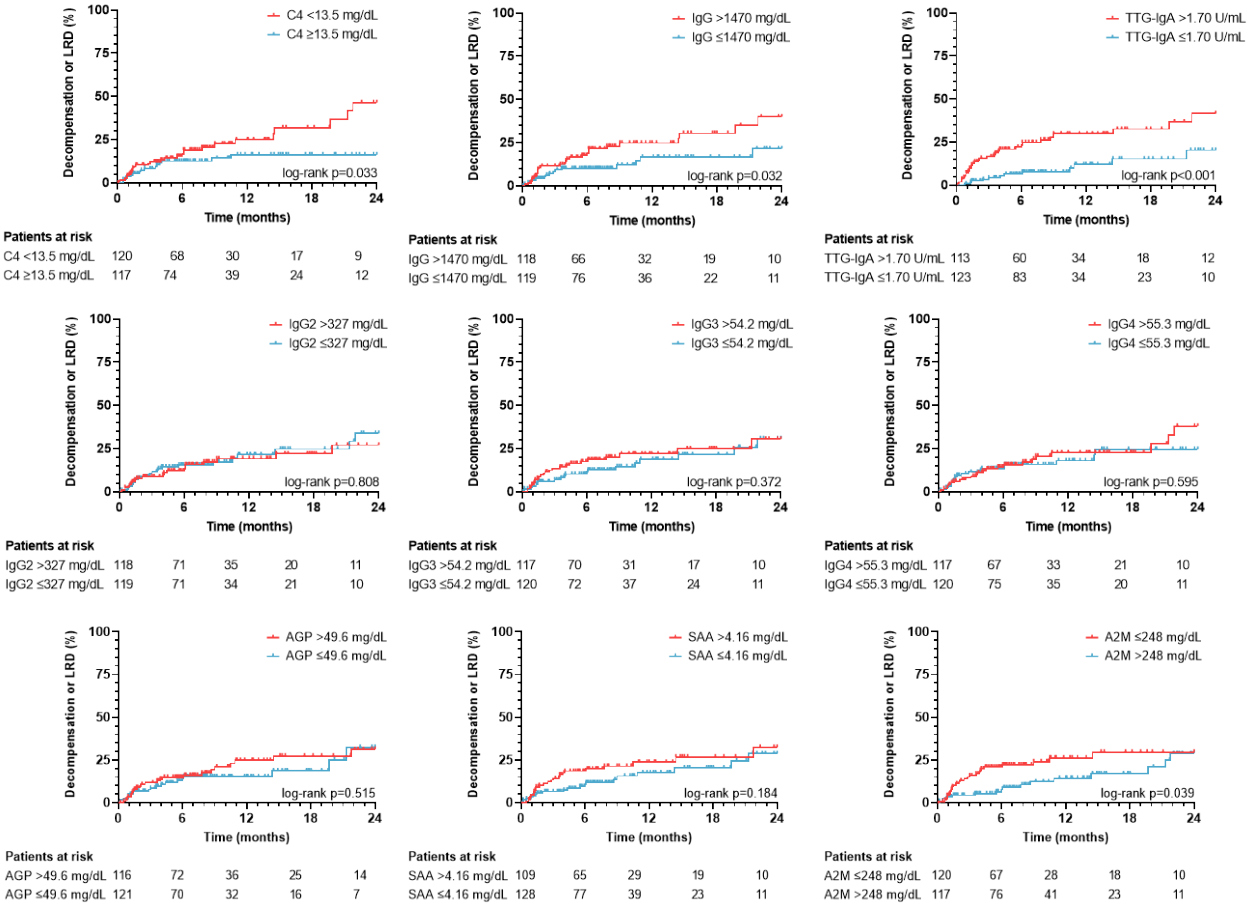


	WBC (G/L)	CRP (mg/dL)	IL-6 (pg/mL)	PCT (ng/mL)	LBP (µg/mL)	C3c (mg/dL)	C4 (mg/dL)	CH50 (U/mL)	IgA (mg/dL)	TTG-IgA (U/mL)	IgM (mg/dL)	IgG (mg/dL)	IgG-1 (mg/dL)	IgG-2 (mg/dL)	IgG-3 (mg/dL)	IgG-4 (mg/dL)	AGP (mg/dL)	SAA (mg/L)	A2M (mg/dL)
WBC (G/L)	1.00	0.17	0.09	0.07	0.15	0.19	0.15	0.13	0.04	0.05	0.04	-0.07	-0.10	0.04	-0.04	0.04	0.07	0.06	-0.02
CRP (mg/dL)	0.17	1.00	0.64	0.49	0.47	-0.04	0.02	0.01	0.35	0.27	0.18	0.16	0.11	0.15	0.21	0.15	0.20	0.42	-0.42
IL-6 (pg/mL)	0.09	0.64	1.00	0.58	0.29	-0.33	-0.18	-0.26	0.50	0.38	0.17	0.31	0.22	0.22	0.25	0.29	0.05	0.18	-0.44
PCT (ng/mL)	0.07	0.49	0.58	1.00	0.12	-0.20	-0.18	-0.17	0.36	0.22	0.09	0.29	0.22	0.21	0.13	0.23	-0.09	0.14	-0.36
LBP (µg/mL)	0.15	0.47	0.29	0.12	1.00	0.47	0.44	0.44	0.12	0.14	-0.15	-0.07	-0.08	-0.03	0.01	0.11	0.56	0.52	-0.04
C3c (mg/dL)	0.19	-0.04	-0.33	-0.20	0.47	1.00	0.66	0.75	-0.26	-0.16	-0.16	-0.28	-0.26	-0.13	-0.17	-0.03	0.44	0.30	0.26
C4 (mg/dL)	0.15	0.02	-0.18	-0.18	0.44	0.66	1.00	0.65	-0.18	-0.15	-0.31	-0.40	-0.43	-0.08	-0.18	-0.05	0.57	0.29	0.15
CH50 (U/mL)	0.13	0.01	-0.26	-0.17	0.44	0.75	0.65	1.00	-0.17	-0.10	-0.35	-0.34	-0.32	-0.14	-0.10	-0.07	0.43	0.34	0.11
IgA (mg/dL)	0.04	0.35	0.50	0.36	0.12	-0.26	-0.18	-0.17	1.00	0.80	0.11	0.51	0.35	0.46	0.28	0.42	-0.15	-0.04	-0.25
TTG-IgA (U/mL)	0.05	0.27	0.38	0.22	0.14	-0.16	-0.15	-0.10	0.80	1.00	0.14	0.43	0.31	0.36	0.28	0.32	-0.11	-0.04	-0.19
IgM (mg/dL)	0.04	0.18	0.17	0.09	-0.15	-0.16	-0.31	-0.35	0.11	0.14	1.00	0.31	0.26	0.26	0.09	0.08	-0.19	-0.12	-0.10
IgG (mg/dL)	-0.07	0.16	0.31	0.29	-0.07	-0.28	-0.40	-0.34	0.51	0.43	0.31	1.00	0.86	0.53	0.40	0.50	-0.29	-0.11	-0.13
IgG-1 (mg/dL)	-0.10	0.11	0.22	0.22	-0.08	-0.26	-0.43	-0.32	0.35	0.31	0.26	0.86	1.00	0.13	0.38	0.31	-0.27	-0.10	-0.07
IgG-2 (mg/dL)	0.04	0.15	0.22	0.21	-0.03	-0.13	-0.08	-0.14	0.46	0.36	0.26	0.53	0.13	1.00	0.23	0.52	-0.17	-0.14	-0.12
IgG-3 (mg/dL)	-0.04	0.21	0.25	0.13	0.01	-0.17	-0.18	-0.10	0.28	0.28	0.09	0.40	0.38	0.23	1.00	0.12	-0.07	0.04	-0.15
IgG-4 (mg/dL)	0.04	0.15	0.29	0.23	0.11	-0.03	-0.05	-0.07	0.42	0.32	0.08	0.50	0.31	0.52	0.12	1.00	-0.06	-0.06	-0.12
AGP (mg/dL)	0.07	0.20	0.05	-0.09	0.56	0.44	0.57	0.43	-0.15	-0.11	-0.19	-0.29	-0.27	-0.17	-0.07	-0.06	1.00	0.53	-0.11
SAA (mg/L)	0.06	0.42	0.18	0.14	0.52	0.30	0.29	0.34	-0.04	-0.04	-0.12	-0.11	-0.10	-0.14	0.04	-0.06	0.53	1.00	-0.10
A2M (mg/dL)	-0.02	-0.42	-0.44	-0.36	-0.04	0.26	0.15	0.11	-0.25	-0.19	-0.10	-0.13	-0.07	-0.12	-0.15	-0.12	-0.11	-0.10	1.00

Statistical analysis: Spearman's correlation coefficients were calculated to assess the association between continous variables.

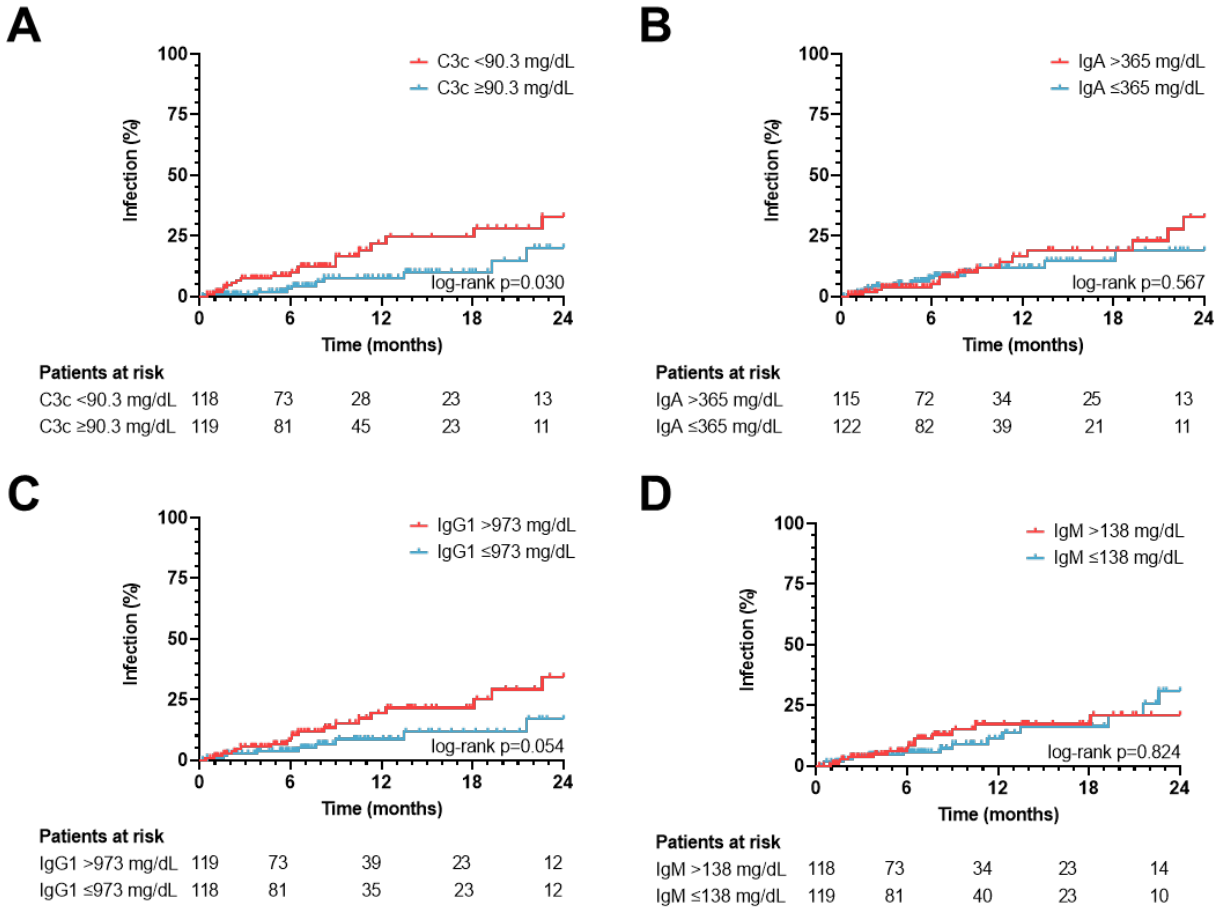
Abbreviations: (A2M) alpha-2-macroglobulin; (AGP) alpha-1-acid glycoprotein; (CRP) C-reactive protein; (C3c) complement C3 component; (Ig) immunoglobulin; (IL-6) interleukin-6; (LBP) lipopolysaccharide binding protein; (PCT) procalcitonin; (SAA) serum amyloid A; (TTG) tissue transglutaminase

Fig. S10. Incidence of first/further decompensation or liver-related death in patients stratified by median complement, immunoglobulin, and acute phase protein levels.



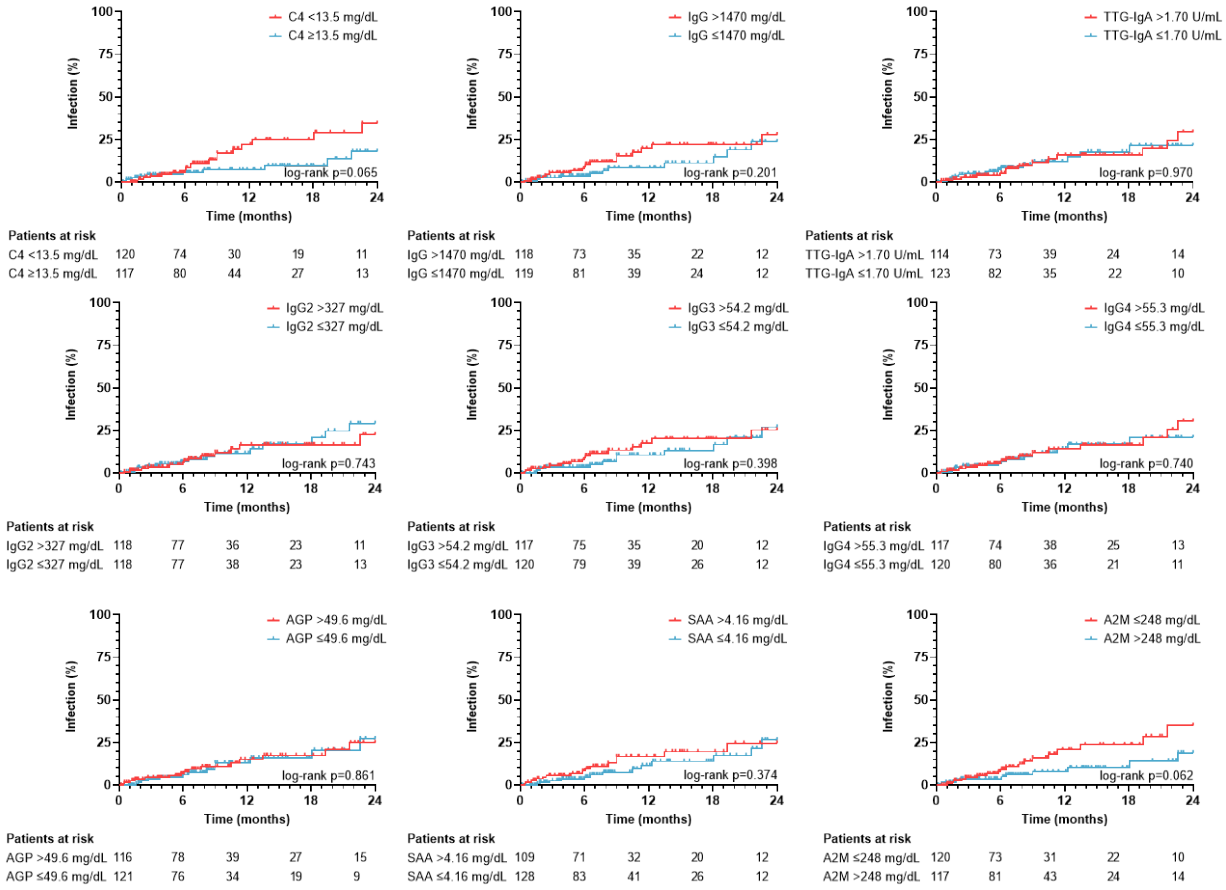
Statistical analysis: The incidence of events in different patient groups was compared by log-rank test. Abbreviations: (Ig) immunoglobulin; (TTG) tissue transglutaminase; (C3c) complement C3 component; (A2M) alpha-2-macroglobulin; (AGP) alpha-1-acid glycoprotein; (SAA) serum amyloid A

Fig. S11. Incidence of infections in patients stratified by median complement and immunoglobulin levels.



Statistical analysis: The incidence of events in different patient groups was compared by log-rank test. Abbreviations: (Ig) immunoglobulin; (C3c) complement C3 component

Fig. S12. Incidence of infections in patients stratified by median complement, immunoglobulin, and acute-phase protein levels.



Statistical analysis: The incidence of events in different patient groups was compared by log-rank test. Abbreviations: (Ig) immunoglobulin; (TTG) tissue transglutaminase; (C3c) complement C3 component; (A2M) alpha-2-macroglobulin; (AGP) alpha-1-acid glycoprotein; (SAA) serum amyloid A

Supplementary tables

Table S1. Patient characteristics.

Parameter	Overall cohort (n=245)	cACLD (n=95)	dACLD (n=150)
Age (years)	57 (50-66)	58 (47-66)	57 (50-66)
Sex (M, %)	161 (66)	64 (67)	97 (65)
Etiology (n, %) - ALD - Viral - ALD + Viral - NASH - Cholestatic - Other	 124 (51) 35 (14) 17 (7) 18 (7) 19 (8) 32 (13)	 30 (32) 24 (25) 3 (3) 17 (18) 12 (13) 9 (10)	 94 (63) 11 (7) 14 (9) 1 (1) 7 (5) 23 (15)
HVPG (mmHg)	17 (12-21)	12 (9-18)	19 (15-22)
MELD Score (points)	11 (9-15)	9 (8-11)	12 (10-16)
Varices (n, %) - None - Small - Large - (Unknown)	 90 (37) 71 (29) 82 (82) 2	 48 (51) 29 (31) 17 (18) 1	 42 (28) 42 (28) 65 (44) 1
CTP stage (n, %) - A - B - C	 126 (51) 93 (38) 26 (11)	 83 (87) 12 (13) 0 (0)	 43 (29) 81 (54) 26 (17)
Bilirubin (mg/dL)	1.19 (0.78-1.98)	0.87 (0.67-1.35)	1.54 (0.96-2.37)
Albumin (g/L)	36.8 (32.9-40.2)	39.9 (36.7-42.4)	35.1 (31.3-37.9)
ASAT (U/L)	40 (29-58)	38 (27-56)	42 (30-58)
WBC (G/L)	4.79 (3.49-6.21)	4.82 (3.31-6.17)	6.25 (4.73-6.25)
CRP (mg/dL)	0.29 (0.11-0.69)	0.16 (0.07-0.36)	0.43 (0.17-1.02)
IL-6 (pg/mL)	9.08 (4.82)	5.45 (3.34-9.08)	12.6 (7.06-22.4)
PCT (ng/mL)	0.10 (0.06-0.16)	0.07 (0.05-0.11)	0.12 (0.07-0.17)
LBP (µg/mL)	6.49 (5.03-8.62)	6.45 (4.91-8.48)	6.51 (5.03-9.24)
C3c (mg/dL)	90.3 (73.1-106)	101 (86.9-114)	81.6 (66.6-98.0)
C4 (mg/dL)	13.5 (9.70-17.2)	14.8 (10.3-19.9)	12.7 (8.98-16.3)
CH50 (U/mL)	52.3 (40.3-60.0)	57.9 (48.9-60.0)	48.0 (34.8-58.7)
IgA (mg/dL)	365 (241-573)	274 (199-424)	467 (289-631)
IgM (mg/dL)	138 (78.3-209)	118 (68.2-175)	151 (89.2-228)
IgG (mg/dL)	1470 (1175-1800)	1340 (1060-1670)	1520 (1270-1873)

IgG1 (mg/dL)	973 (740-1275)	882 (684-1100)	1030 (784-1312)
IgG2 (mg/dL)	327 (243-466)	302 (220-412)	342 (252-526)
IgG3 (mg/dL)	54.2 (35.7-82.2)	47.8 (31.7-72.1)	58.0 (37.7-86.6)
IgG4 (mg/dL)	55.3 (25.5-113)	44.8 (21.6-94.4)	60.0 (33.2-133)
TTG-IgA (U/mL)	1.70 (1.00-2.65)	1.20 (1.00-2.20)	1.20 (2.05-3.03)
AGP (mg/dL)	49.6 (35.4-61.7)	51.3 (40.7-62.3)	47.0 (33.4-61.1)
SAA (mg/L)	4.16 (4.16-7.26)	4.16 (4.16-7.01)	4.16 (4.16-7.49)
A2M (mg/dL)	248 (198-301)	286 (228-331)	228 (185-264)
Rifaximin/Antibiotic prophylaxis (n, %)†	23 (9)	0 (0)	23 (15)

† Antibiotic therapy regimens: n=20 rifaximin monotherapy, n=2 rifaximin and norfloxacin, n=1 norfloxacin.

Abbreviations: (A2M) alpha-2-macroglobulin; (AGP) alpha-1-acid glycoprotein; (ALD) alcohol-related liver disease; (c/dACLD) compensated/decompensated advanced chronic liver disease; (C3c) complement C3 component; (CRP) C-reactive protein; (HVPG) hepatic venous pressure gradient; (Ig) immunoglobulin; (IL-6) interleukin-6; (LBP) lipopolysaccharide binding protein; (M) male sex; (MELD) Model of End Stage Liver Disease; (NASH) non-alcoholic steatohepatitis; (PCT) procalcitonin; (SAA) serum amyloid A; (TTG) tissue transglutaminase

Table S2. The incidence of decompensation/liver-related death compared by log-rank test in patients stratified by median complement, immunoglobulin, or acute-phase protein levels.

Parameter	Cut-off	HR (log rank)	95% CI	P-value
C3c (mg/dL)	<90.3	2.58	1.42-4.70	**0.003**
C4 (mg/dL)	<13.5	1.95	1.07-3.55	**0.033**
IgA (mg/dL)	>365	3.61	1.98-6.58	**<0.001**
TTG-IgA (U/mL)	>1.70	3.12	1.71-5.69	**<0.001**
IgM (mg/dL)	>138	1.61	0.88-2.93	0.124
IgG (mg/dL)	>1470	1.96	1.08-3.57	**0.032**
IgG1 (mg/dL)	>973	1.89	1.04-3.44	**0.043**
IgG2 (mg/dL)	>327	0.89	0.49-1.62	0.709
IgG3 (mg/dL)	>54.2	1.31	0.72-2.39	0.372
IgG4 (mg/dL)	>55.3	1.18	0.65-2.14	0.595
AGP (mg/dL)	>49.6	1.22	0.67-2.22	0.515
SAA (mg/L)	>4.16	1.50	0.82-2.73	0.184
A2M (mg/dL)	≤248	1.89	1.04-3.44	**0.039**

Statistical analysis: The incidence of events in different patient groups was compared by log-rank test. P-values <0.05 are indicated in bold. Abbreviations: (A2M) alpha-2-macroglobulin; (AGP) alpha-1-acid glycoprotein; (C3c) complement C3 component; (FU) follow-up; (Ig) immunoglobulin; (SAA) serum amyloid A; (TTG) tissue transglutaminase

Table S3. The incidence of infections compared by log-rank test in patients stratified by median complement, immunoglobulin, or acute-phase protein levels.

Parameter	Cut-off	HR (log rank)	95% CI	P-value
C3c (mg/dL)	<90.3	2.34	1.12-4.92	**0.030**
C4 (mg/dL)	<13.5	2.03	0.97-4.27	0.065
IgA (mg/dL)	>365	1.24	0.59-2.61	0.567
TTG-IgA (U/mL)	>1.70	1.01	0.48-2.13	0.970
IgM (mg/dL)	>138	1.09	0.52-2.29	0.824
IgG (mg/dL)	>1470	1.63	0.78-3.42	0.201
IgG1 (mg/dL)	>973	2.14	1.02-4.48	0.054
IgG2 (mg/dL)	>327	0.88	0.42-1.85	0.743
IgG3 (mg/dL)	>54.2	1.38	0.66-2.89	0.398
IgG4 (mg/dL)	>55.3	1.13	0.54-2.38	0.740
AGP (mg/dL)	>49.6	1.07	0.51-2.24	0.861
SAA (mg/L)	>4.16	1.40	0.66-2.94	0.374
A2M (mg/dL)	≤248	2.05	0.98-4.31	0.062

Statistical analysis: The incidence of events in different patient groups was compared by log-rank test. P-values <0.05 are indicated in bold. Abbreviations: (A2M) alpha-2-macroglobulin; (AGP) alpha-1-acid glycoprotein; (C3c) complement C3 component; (FU) follow-up; (Ig) immunoglobulin; (SAA) serum amyloid A; (TTG) tissue transglutaminase

Supplementary reference

1. *EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma.* J Hepatol, 2018. **69**(1): p. 182-236.