COVER PAGE

Supplemental Materials

Title: Complement C3 Facilitates Stratification of Stages of Chronic Hepatitis B and Signifies Development of Acute-on-Chronic Liver Failure in Acute Decompensated Cirrhosis

Chong Chen, Zhu Yuan, Weixia Li, Ling Fei, Liujuan Ji, Qin Huang, Shuye Zhang, Liang Chen

Chong Chen and Zhu Yuan are co-first authors.

Liang Chen and Shuye Zhang are joint corresponding Authors.

Department of Infectious Diseases, Shanghai Public Health Clinical Centre, Fudan University, Shanghai 201508, China

Chong Chen, Weixia Li & Qin Huang

Department of Endocrinology, Jinshan Branch of Shanghai Sixth People's Hospital, Shanghai 201599, China

Zhu Yuan

Department of Hepatology, Shanghai Public Health Clinical Centre, Fudan University, Shanghai 201508, China

Ling Fei & Liang Chen

Department of Severe Liver Disease, Shanghai Public Health Clinical Centre, Fudan University, Shanghai 201508, China

Liujuan Ji

Clinical Center for BioTherapy, Zhongshan Hospital, Fudan University, Shanghai 200031, China.

Shuye Zhang

Corresponding Authors: Prof Liang Chen and Prof Shuye Zhang.

Shanghai Public Clinical Centre, 2901 Caolang Road, Jin-Shan District, Shanghai 201508, P.R. China and Clinical Center for BioTherapy, Zhongshan Hospital, Fudan University. No.180 Fengling Road, Xuhui District, Shanghai 200031, China.

E-mail: chenliang@shphc.org.cn and shuye_zhang@fudan.edu.cn. Tel: +86(21)57248785; Fax: +86(21)57248782.

Definition of ACLF Grades

ACLF grades were defined according to the COSSH (Chinese group on the study of severe hepatitis B) criteria (*Gut. 2018;67:2181-2191*). Detailed content is as follows:

ACLF grade 1: including: (a) patients with kidney failure alone; (b) patients with single liver failure with an INR ≥1.5 and/or kidney dysfunction and/or HE grade I or II; (c) patients with single type of organ failure of the coagulation, circulatory or respiratory systems and/or kidney dysfunction and/or HE grade I or II and (d) patients with cerebral failure alone plus kidney dysfunction.

ACLF grade 2: Patients with failures of two organ systems.

ACLF grade 3: Patients with failures of 3 or more organ systems.

Supplementary table 1. Baseline characteristics, laboratory parameters of 223 HBV-C-ACLF patients.

Parameters	Total ACLFs (n=223)	ACLF-1 (n=133)	ACLF-2 (n=64)	ACLF-3 (n=26)	*P* value
Age (y)	52 (45-62)	52 (46-52)	50 ± 11.5	57 ± 12	0.060
Gender (male, %)	170 (76.2)	99 (74.4)	47 (73.4)	24 (92.3)	0.121
HBe Ag (+), n (%)	63 (28.3)	39 (29.3)	17 (26.6)	7 (26.9)	0.910
HBV DNA, (IU/mL)					
< 500	54 (24.2)	34 (25.6)	13 (20.3)	7 (26.9)	0.262
$500\text{-}10^5$	108 (48.4)	60 (45.1)	32 (50.0)	16 (61.5)	
$> 10^5$	61 (27.4)	39 (29.3)	19 (29.7)	3 (11.5)	
Clinical complications at enrollment, n (%)					
Ascites	171 (76.7)	89 (66.9)	57 (89.1)	25 (96.2)	< 0.001
Bacterial infection	68 (30.5)	9 (6.8)	33 (51.6)	22 (84.6)	< 0.001
Hepatic encephalopathy	97 (43.5)	37 (27.8)	42 (65.6)	18 (69.2)	< 0.001

Gastrointestinal haemorrhage	7 (3.1)	4 (3.0)	1 (1.6)	2 (7.7)	0.316
Laboratory parameters					
ALT, U/L	139 (48-473)	117 (41-449)	174 (63-750)	90 (62-285)	0.086
AST, U/L	120 (62-286)	114 (58-258)	156 (83-477)	120 (71-237)	0.087
Tbil, mg/dL	21.2 (11.5-29.6)	15.5 (9.1-25.6)	24.7 (18.3-32.0)	30.5 (19.5-41.1)	< 0.001
Albumin, g/L	31.4 (28.2-35.0)	31.8 ± 5.3	32.2 ± 4.5	30.6 (26.8-33.6)	0.331
INR	2.14 (1.63-2.95)	1.72 (1.43-2.12)	3.56 ± 1.61	2.87 (2.59-3.94)	< 0.001
Creatinine, μmol/L	63.9 (50.4-79.8)	61.0 (48.8-73.4)	61.2 (50.3-77.2)	142 (71.0-260.0)	< 0.001
Urea, mmol/L	5.09 (3.44-7.03)	4.85 (3.47-6.33)	5.31 ± 3.14	10.14 (6.83-13.98)	< 0.001
Glucose, mmol/L	6.83 (5.03-8.56)	6.65 (4.86-8.95)	7.34 (5.18-8.48)	6.52 (5.64-8.34)	0.838
WBC, 10^9/L	6.08 (4.40-8.35)	5.31 (3.63-7.53)	8.06 ± 4.51	8.07 (5.63-12.77)	< 0.001
Haemoglobin, g/L	115 ± 22	116 ± 21	115 ± 22	109 (89-118)	0.029
Platelet, 10^9/L	88 (51-135)	107 ± 66	87 ± 40	61 (44-107)	< 0.001

C3, g/L	0.36 (0.25-0.55)	0.53 ± 028	0.33 ± 0.15	0.26 ± 0.11	< 0.001
Prognostic score					
COSSH-ACLFs	7.8 (6.5-9.9)	6.7 (5.8-7.5)	9.9 (8.8-10.6)	11.8 (10.5-14.3)	< 0.001
COSSH ACLF IIs	7.3 (6.5-8.3)	6.7 (6.3-7.4)	8.4 (7.5-8.9)	9.6 (8.1-10.3)	< 0.001
Poor prognosis					
28-days mortality	114 (51.1)	42 (31.6)	48 (75.0)	24 (92.3)	< 0.001
Liver transplantation	22 (9.9)	9 (6.8)	11 (17.2)	2 (7.7)	0.066

NOTE: The groups of patients with ACLF of cirrhosis were named as HBV-C-ACLF. Data are presented as the median (Q1-Q3) or the number of patients (%). The continuous data between two groups were compared by t-test or Mann-Whitney-test.The categorical data between two groups were compared with Chi-square test or Fisher's exact test.

Abbreviations: ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Tbil, total bilirubin; INR, international normalised ratio; WBC, white blood cells; C3, complement component 3; COSSH, Chinese group on the study of severe hepatitis B.

Supplementary table 2. Comparison of the characteristics between patients with and without poor prognosis among HBV-C-ACLFs.

Parameters	Patients with poor prognosis (n=136)	Patients without poor prognosis (n=87)	P value
Age (y)	52 (44-61)	51 (45-64)	0.503
Gender (male, %)	108 (79.4)	62 (71.2)	0.109
HBe Ag (+), n (%)	38 (27.9)	25 (28.7)	0.508
HBV DNA, (IU/mL)			
< 500	33 (24.3)	21 (24.1)	0.651
500-10^5	63 (46.3)	45 (51.7)	
> 10^5	40 (29.4)	21 (24.1)	
Clinical complications at enrollment, n (%)			
Ascites	120 (88.2)	51 (58.6)	< 0.001
Bacterial infection	53 (39.0)	15 (17.2)	< 0.001
Hepatic encephalopathy	87 (64.0)	10 (11.5)	< 0.001
Gastrointestinal haemorrhage	7 (5.1)	0	< 0.05
Laboratory parameters			
ALT, U/L	140 (49-551)	137 (47-425)	0.579
AST, U/L	131 (75-341)	114 (57-225)	0.058

Tbil, mg/dL	25.0 ± 13.1	15.3 ± 9.9	< 0.001
Albumin, g/L	31.5 ± 5.0	32.0 ± 4.9	0.579
INR	2.63 (1.95-3.58)	1.63 (1.39-1.91)	0.544
Creatinine, μmol/L	64.6 (51.7-83.9)	61.9 (48.1-77.4)	0.102
Urea, mmol/L	5.58 (3.71-8.07)	4.47 (3.29-5.82)	< 0.01
Glucose, mmol/L	7.0 (5.23-8.39)	6.62 (4.80-9.15)	0.544
WBC, 10^9/L	6.98 (4.77-9.64)	5.26 (3.42-6.94)	< 0.001
Haemoglobin, g/L	112 ± 22	119 ± 20	< 0.05
Platelet, 10^9/L	87 ± 44	114 ± 72	< 0.01
C3, g/L	0.33 (0.23-0.43)	0.54 (0.31-0.71)	< 0.001
Prognostic score			
COSSH-ACLFs	9.3 (7.8-10.7)	6.4 (5.5-7.0)	< 0.001
COSSH ACLF IIs	8.1 (7.3-8.9)	6.5 (6.1-7.1)	< 0.001

NOTE: The groups of patients with ACLF of cirrhosis were named as HBV-C-ACLF. The poor prognosis of patients was defined as mortality or liver transplantation within 28 days after admission. Data are presented as the median (Q1-Q3) or the number of patients (%). The continuous data between two groups were compared by t-test or Mann-Whitney-test.The categorical data between two groups were compared with Chi-square test or Fisher's exact test.

Abbreviations: ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Tbil, total bilirubin; INR, international normalised ratio; WBC, white blood cells; C3, complement component 3; COSSH, Chinese group on the

study of severe hepatitis B.

Supplementary table 3. Comparison of the characteristics between discovery and validation cohorts in the patients with acute decompensated cirrhosis.

Parameters	Discovery cohort (n=119)	Validation cohort (n=86)	*P* value
Age (y)	59 (48-71)	56 (47-64)	< 0.05
Gender (male, %)	66 (55.5)	55 (64.0)	0.251
HBe Ag (+), n (%)	45 (37.8)	26 (30.2)	0.299
HBV DNA, (IU/mL)			
< 500	43 (36.1)	34 (39.5)	0.767
500-10^5	46 (38.7)	29 (33.7)	
> 10^5	30 (25.2)	23 (26.7)	
Clinical complications at enrollment, n (%)			
Ascites	84 (70.6)	59 (68.6)	0.761
Bacterial infection	25 (21.8)	17 (19.8)	0.863
Hepatic encephalopathy	30 (25.2)	16 (18.6)	0.310
Gastrointestinal haemorrhage	4 (3.4)	1 (1.2)	0.401
Laboratory parameters			
ALT, U/L	102 (33–211)	91 (26-178)	0.213
AST, U/L	89 (25–177)	67 (18-142)	0.071
Tbil, mg/dL	3.92 ± 2.20	4.98 (2.21-8.10)	0.124
Albumin, g/L	30.9 (27.6-35.8)	31.0 (26.8-39.3)	0.780
INR	1.42 (1.30 ± 1.62)	1.39 (1.22-1.54)	0.122

Creatinine, μmol/L	60.1 (45.9-72.3)	70.0 (52.1-90.1)	0.080
Urea, mmol/L	3.10 (2.70-4.92)	3.33 (2.89-5.44)	0.335
Glucose, mmol/L	6.09 (4.91-8.07)	5.90 (5.10-7.89)	0.761
WBC, 10^9/L	5.61 (4.10-7.34)	5.71 (4.22-7.68)	0.672
Haemoglobin, g/L	109 ± 16	106 ± 14	0.090
Platelet, 10^9/L	94 (62-139)	101 (70-151)	0.553
C3, g/L	0.72 ± 0.25	0.63 ± 0.29	0.489
Prognostic score			
COSSH-ACLFs	5.8 (4.8-6.1)	5.8 (5.2-6.2)	0.317
COSSH ACLF IIs	5.2 (4.8-6.0)	5.1 (4.6-6.4)	0.998

NOTE: Data are presented as the median (Q1-Q3) or the number of patients (%). The continuous data between two groups were compared by t-test or Mann-Whitney-test. The categorical data between two groups were compared with Chi-square test or Fisher's exact test. (**NOTICE:** The validation cohort was screened and enrolled between January 2022 to June 2022 from the Department of Hepatology and Department of Severe Liver Disease, Shanghai Public Health Clinical Centre, Fudan University, Shanghai, China. 86 patients with acute decompensated cirrhosis were qualified in the validation cohort.)

Abbreviations: AD, acute decompensation; ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Tbil, total bilirubin; INR, international normalised ratio; PT, prothrombin; WBC, white blood cells; C3, complement component 3; COSSH, Chinese group on the study of severe hepatitis B.
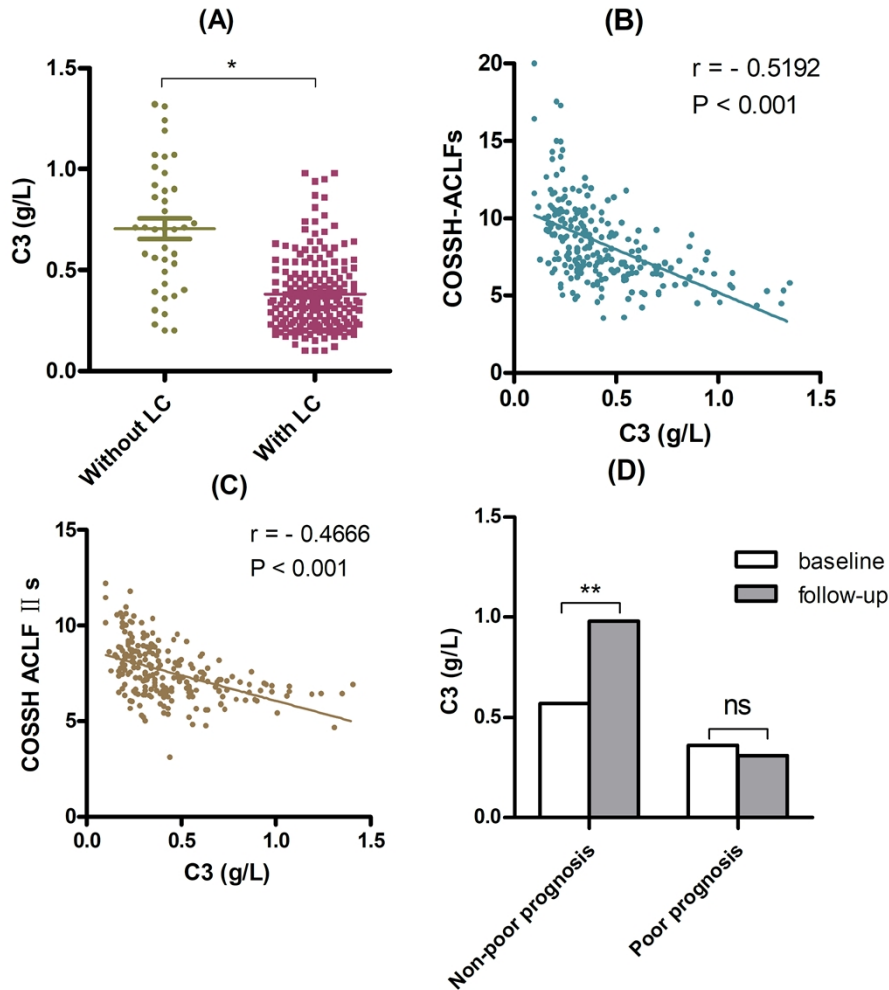
Supplementary figure 1. Association of liver complications, ACLF scores and prognosis of disease with C3 levels in patients with HBV-C-ACLF.

(A) Patients with liver complications had significant lower C3 levels; **(B)** A significant negative correlation between C3 levels and COSSH-ACLFs; **(C)** A significant negative correlation between C3 levels and COSSH ACLF IIs; **(D)** C3 levels variation by clinical outcomes of disease in patients with HBV-C-ACLF.

NOTE: The groups of patients with ACLF of cirrhosis were named HBV-C-ACLF. The poor prognosis of patients was defined as mortality or liver transplantation within

28 days after admission. *, < 0.05; **, < 0.01; ns, not significant.

Abbreviations: C3, complement component 3; LC, liver complication; COSSH, Chinese group on the study of severe hepatitis B.
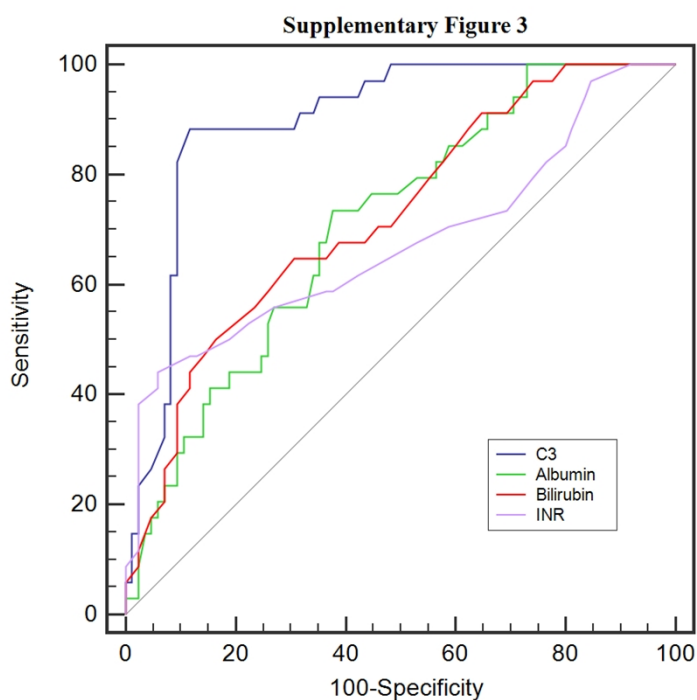
Supplementary Figure 2
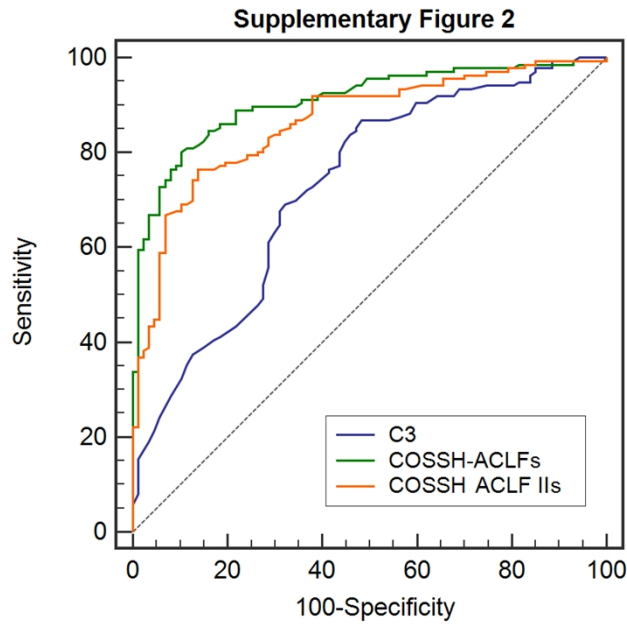
Predictors	Poor prognosis of HBV-C-ACLFs	
	auROC	95 CI%
C3	0.73	0.66-0.80
COSSH-ACLFs	0.91	0.87-0.95
COSSH ACLF IIs	0.86	0.82-0.91
C3 *vs* COSSH-ACLFs	*P* = 0.0001	
C3 *vs* COSSH ACLF IIs	*P* = 0.0002	

Supplementary figure 2. ROC curves for predicting the poor prognosis of 223 HBV-C-ACLF patients.

NOTE: The groups of patients with ACLF of cirrhosis were named HBV-C-ACLF. The poor prognosis of patients was defined as mortality or liver transplantation within 28 days after admission. AuROCs for different models were calculated and compared using the Z test (Delong's method).

Abbreviations: C3, complement component 3; COSSH, Chinese group on the study of severe hepatitis B; ACLF, acute-on-chronic liver failure; auROC, the area under the receiver operating characteristic.

Supplementary figure 3. ROC curves for development of ACLF in 119 HBV-C-AD patients.

Predictors	Development of ACLF in patients with decompensated cirrhosis	
	auROC	95 CI%
C3	0.90	0.84-0.96
Bilirubin	0.72	0.62-0.82
INR	0.68	0.56-0.80
Albumin	0.71	0.61-0.81
C3 *vs* Bilirubin	*P* = 0.0053	
C3 *vs* INR	*P* = 0.0024	
C3 *vs* Albumin	*P* = 0.0026	

Note: Follow up the HBV-C-AD patients to establish whether they developed ACLF within 28 days after admission. AuROCs for different models were calculated and compared using the Z test (Delong's method).

Abbreviations: C3, complement component 3; AD, acute decompensation; ACLF, acute-on-chronic liver failure; INR, international normalised ratio; auROC, the area under the receiver operating characteristic.