Role of precipitants in transition of acute decompensation to acute-on-chronic liver failure in patients with HBV-related cirrhosis

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Supplementary materials and methods

Definition

The diagnosis criteria for ACLF

ACLF was diagnosed following the European Association for the Study of the Liver (EASL) consortium definition. [1]. Accordingly, ACLF grade 1 (ACLF-1) was defined by single kidney failure (serum creatinine ≥2 mg/dL) or single cerebral failure (grade III-IV HE for brain based on West Haven criteria) with renal dysfunction (serum creatinine ranging from 1.5 to 1.9 mg/dL) or other single organ failure (serum bilirubin ≥12 mg/dL for liver; INR [international normalized ratio] ≥ 2.5 for coagulation; vasopressors to maintain arterial pressure for circulation; PaO2/FiO2 ≤200 or SpO2/FiO2 ≤214 for respiration) with renal dysfunction and/or grade I-II HE. ACLF grade 2 (ACLF-2) and ACLF grade 3 (ACLF-3) were defined by the presence of 2 or no less than 3 organ failures, respectively.

The diagnosis criteria of bacterial infections of different types

(1) Pneumonia: radiological evidence of new pulmonary infiltrate with one of the following: (a) any respiratory symptoms (such as cough, sputum, dyspnea, or pleuritic pain), (b) any findings on auscultation (rales or crepitation) or fever/chills/shivering, or white blood cell (WBC) count >10,000/mm3 or <4,000/mm3;

(2) Spontaneous bacterial peritonitis (SBP): ascitic fluid polymorphonuclear (PMN) cells >250/mL, with or without positive ascites culture[2];

(3) Bacteremia: positive blood cultures with or without a source of infection;

(4) Urinary tract infection: urine WBC >15/high power field (HPF) with positive culture and symptom of urinary irritation;

(5) Skin infection: fever and leukocytosis with cellulitis;

(6) other bacterial infections, including infection of biliary tract, liver abscess, testicular infection,

purulent meningitis, intra-abdominal infection, and other clear infection sites.

Study setting and locations

Names and locations of centers (tertiary hospitals) in the derivation and validation cohorts[3, 4]:

1. Ren Ji Hospital, School of Medicine, Shanghai, Jiao Tong University, Shanghai, China;

2. Beijing Ditan Hospital, Capital Medical University, Beijing, China

3. Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China

4. Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

5. Xiangya Hospital, Central South University, Changsha, China

6. Nanfang Hospital, Southern Medical University, Guangzhou, China

7. Taihe Hospital, Hubei University of Medicine, Shiyan, China

8. Shanghai Public Health Clinical Centre, Fudan University, Shanghai, China

9. The Second Hospital of Shandong University, Jinan, China

10. The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China

11. Henan Provincial People's Hospital, Zhengzhou, China

12. The First Hospital of Jilin University, Changchun, China

13. The First Affiliated Hospital of School of Medicine, Zhejiang University, Hangzhou, China

14. Fuzhou General Hospital of Nanjing Military Command, Fujian, China

15. Affiliated Hospital of Logistics University of People's Armed Police Force, Tianjin, China

No.1-12,14,15 centers participated in the derivation cohort, No.1-13 centers participated in the validation cohort in the Chinese Acute-on-Chronic Liver Failure (CATCH-LIFE) study.

The management of patients

In the study, each patients received standard therapies targeting at etiology, precipitants or complications.

Patients with detectable HBV DNA immediately started a long course of nucleoside analogs treatment (entecavir 0.5 mg/day or tenofovir 300 mg/day). Patients with active alcohol assumption were required to abstain alcohol. Patients who were considered with drug induced liver injury (DILI) were required to stop using the suspicious hepatotoxic drugs. Standard etiological treatments of other causes of CLDs in the study, including HCV, NAFLD, autoimmune liver diseases, Wilson disease, et cetera, were given to each relevant patient as suggested.

The management of decompensation complications were as follow: 1) Diagnostic abdominocentesis were performed in patients with ascites. Patients with uncomplicated moderate ascites were treated with aldosterone antagonist and/or furosemide. For those with large or refractory ascites, paracentesis combining with intravenous albumin, or tolvaptan, was used. For patients complicated with spontaneous bacterial peritonitis (SBP), an empirical antibiotic therapy, mainly 3rd generation cephalosporin, β-lactam/β-lactamase inhibitors or Carbapenem, with intravenous albumin, was initiated immediately. For patients complicated with acute kidney injury (AKI), potential causes were investigated and removed or corrected as possible. And terlipressin plus albumin was used for HRS-AKI; 2) patients with acute gastrointestinal bleeding were treated with somatostatin, proton pump inhibitors (PPI) and antibiotic prophylaxis and endoscopy was performed as early as possible. For those with uncontrolled hemorrhage, an urgent therapeutic endoscopy, Sengstaken-Blackmore tube or TIPS was performed; 3) patients with hepatic encephalopathy were treated with oral lactulose and intravenous L-ornithine aspartate, and

potential causes were investigated and removed or corrected as possible; 4) patients with bacterial infections other than SBP received empirical antibiotic therapies. Organ supporting, including mechanical ventilation, volume replacement and/or use of vasoactive agents, or renal replacement, was performed for patients with organ failure. Extracorporeal liver supporting was selectively used for patients with liver failure (defined as TB > 10 mg/dL and INR >1.5), brain failure (HE over III grade) or renal failure[5].

Generally, a standard for recommendation for liver transplantation was proposed for the study: 1) patients with decompensated cirrhosis and recurrent or uncontrolled complications following standard therapies, for instance, refractory ascites, recurrent variceal hemorrhage, and with a MELD Score >15; or 2) patients with acute-on-chronic liver failure (ACLF) who were unresponsive to standard therapies, and often with extracorporeal liver supporting. The priority of patients in the waiting list of liver transplantation was determined by MELD-Na score.[6, 7] Moreover, patients with at least 3 organ failures but without circulation or lung failure were given higher priority in the waiting list of liver transplantation.

Supplementary tables

Table S1. Bacterial infection types in patients with ACLF at enrollment, ACLF development and no ACLF development patients.

Identified bacterial infection types	ACLF (n= 308)	Pre-ACLF (n= 137)	UDC (n= 380)	SDC (n= 728)	P value
Pneumonia	35 (11.4)	15 (10.9)	18 (4.7)	40 (5.5)	<0.001
Urinary tract infection	1 (0.3)	0 (0.0)	1 (0.3)	3 (0.4)	0.881
Spontaneous bacterial peritonitis	30 (9.7)	10 (7.3)	16 (4.2)	32 (4.4)	0.003
Bacteremia	9 (2.9)	5 (3.6)	3 (0.8)	6 (0.8)	0.007
Cellulitis	0 (0.0)	0 (0.0)	2 (0.5)	1 (0.1)	0.367
Other infections	7 (2.3)	1 (0.7)	4 (1.1)	12 (1.6)	0.507

Note: Other infections: including infection of biliary tract, liver abscess, testicular infection, purulent meningitis, intra-abdominal infection, and other clear infection sites.

Table S2. Comparison of laboratory indicators and severity scores in pre-ACLF patients at enrollment and at development time point.

	Pre-ACLF (n = 94)		p value
	At Enrollment	**At ACLF development**	
Laboratory data, median (IQRs)			
TB (mg/dL)	19.36 [9.46, 27.39]	20.76 [13.49, 29.71]	0.107
INR	2.01 [1.73, 2.28]	2.55 [1.89, 2.75]	<0.001
Cr (mg/dL)	0.79 [0.68, 1.01]	0.90 [0.70, 1.20]	0.015
BUN (mEq/L)	4.90 [3.51, 6.90]	5.40 [3.68, 9.17]	0.108
Albumin (g/L)	30.40 [27.70, 34.09]	31.59 [29.10, 34.71]	0.036
Hemoglobin (g/L)	119.00 [99.00, 133.00]	104.00 [85.00, 120.50]	<0.001
WBC (10^9/L)	6.02 [4.50, 8.01]	6.10 [4.05, 8.47]	0.984
PLT (10^9/L)	79.00 [54.00, 108.00]	65.50 [39.75, 92.75]	0.005
Sodium (mEq/L)	136.50 [132.90, 139.50]	135.40 [132.00, 138.55]	0.171
AST/ALT	1.15 [0.83, 1.59]	1.43 [1.07, 2.11]	0.001
Prealbumin, mg/L	41.65 [20.75, 59.00]	33.73 [1.54, 46.75]	0.013
Procalcitonin (ng/ml)	0.75 [0.43, 1.23]	0.77 [0.54, 1.15]	0.443
Liver function, mean (SD)			
MELD	25.00 [22.00, 27.00]	29.00 [26.00, 31.00]	<0.001
MELD-sodium	26.00 [24.00, 30.00]	31.00 [27.00, 34.50]	<0.001
CLIF-C AD	53.54 [47.83, 58.50]	58.50 [51.61, 65.65]	<0.001
CLIF-C ACLF	38.90 [35.38, 42.12]	44.27 [38.97, 49.02]	<0.001

Statistical analysis was performed by a paired Student t test.

Table S3. Association between precipitation events and ACLF development during hospitalization in HBV related cirrhotic patients with acutely decompensation.

Precipitation events, n (%)	ACLF development (N= 94)	No ACLF development (N= 876)	p value
Intra-hepatic			
Hepatitis B flare with HBV reactivation	18 (19.1)	37 (4.2)	<0.001
Spontaneous hepatitis B flare with high HBV-DNA load	13 (13.9)	48 (5.5)	0.003
Spontaneous hepatitis B flare with low HBV-DNA load	14 (14.9)	74 (8.5)	0.062
No hepatitis B flare with HBV reactivation	10 (10.6)	77 (8.9)	0.426
No hepatitis B flare with high HBV load	6 (6.4)	71 (8.8)	0.400
No hepatitis B flare with low HBV load	19 (20.2)	285 (32.5)	<0.001
Superimposed infection on HBV	7 (7.4)	20 (2.3)	0.010
Hepatotoxic drugs	8 (8.5)	35 (4.0)	0.079
Active alcohol intaking	5 (5.3)	39 (4.5)	0.902
Extra-hepatic			
Bacterial infection	54 (57.4)	208 (23.7)	<0.001
Variceal bleeding	8 (8.5)	179 (20.4)	0.008
Portal vein thrombosis	5 (5.3)	78 (8.9)	0.324
Surgery	2 (2.1)	15 (1.7)	1.000

Table S4. Prevalence of precipitant events in different ACLF grade at enrollment

Precipitants, n (%)	Grade 1 (n= 43)	Grade 2 (n= 135)	Grade 3 (n= 19)	P value
Hepatitis B flare with HBV reactivation	2 (4.7)	18 (13.3)	2 (10.5)	0.288
Spontaneous hepatitis B flare with high HBV-DNA load	4 (9.3)	18 (13.3)	1 (5.3)	0.509
Superimposed infection on HBV	3 (7.0)	12 (8.9)	1 (5.3)	0.823
Hepatotoxic drugs	2 (4.7)	8 (5.9)	0 (0.0)	0.539
Active alcohol intaking	1 (2.3)	13 (9.6)	1 (5.3)	0.267
Bacterial infection	21 (48.8)	67 (49.6)	8 (42.1)	0.828
Variceal bleeding	5 (11.6)	4 (3.0)	1 (5.3)	0.079
Portal vein thrombosis	1 (2.3)	2 (1.5)	0 (0.0)	0.787
Surgery	0 (0.0)	1 (0.5)	0 (0.0)	0.777

Table S5. Prevalence of precipitant events in liver, coagulation and extra-hepatic organ failure of ACLF at enrollment

Precipitants, n (%)	Liver failure (n= 177)	Coagulation (n= 139)	Both* (n= 133)	Extra-hepatic organ failure (n= 53)
Hepatitis B flare with HBV reactivation	22 (12.4)	15 (10.8)	15 (11.3)	6 (11.3)
Spontaneous hepatitis B flare with high HBV-DNA load	22 (12.4)	19 (13.7)	18 (13.5)	2 (3.8)
Superimposed infection on HBV	16 (9.0)	12 (8.6)	12 (9.0)	3 (5.7)
Hepatotoxic drugs	10 (5.6)	6 (4.3)	6 (4.5)	2 (3.8)
Active alcohol intaking	15 (8.5)	12 (8.6)	12 (9.0)	3 (5.7)
Bacterial infection	87 (49.2)	65 (46.8)	63 (47.4)	27 (50.9)
Variceal bleeding	5 (2.8)	4 (2.9)	3 (2.3)	7 (13.2)
Portal vein thrombosis	2 (1.1)	2 (1.4)	1 (0.8)	1 (1.9)
Surgery	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)

*Both liver and coagulation failure.

Table S6. Prevalence of precipitant events in changed ACLF grades at day 4 and day 7

	Day4					Day7				
	Grade0 (n= 45)	Grade1 (n= 30)	Grade2 (n= 87)	Grade3 (n= 23)	P value	Grade 0 (n= 55)	Grade1 (n= 24)	Grade2 (n= 76)	Grade3 (n= 22)	P value
ACLF grade at enrollment					<0.001					<0.001
Grade 1	18 (40.0)	20 (66.7)	4 (4.6)	0 (0.0)		19 (34.5)	16 (66.7)	4 (5.3)	2 (9.1)	
Grade 2	23 (51.1)	10 (33.3)	79 (90.8)	14 (60.9)		30 (54.5)	8 (33.3)	68 (89.5)	16 (72.7)	
Grade 3	4 (8.9)	0 (0.0)	4 (4.6)	9 (39.1)		6 (10.9)	0 (0.0)	4 (5.3)	4 (18.2)	
Precipitant events										
Hepatitis B flare with HBV reactivation	3 (6.7)	4 (13.3)	11 (12.6)	2 (8.7)	0.703	5 (9.1)	2 (8.3)	11 (14.5)	1 (4.5)	0.515
Spontaneous hepatitis B flare with high HBV-DNA load	1 (2.2)	5 (16.7)	13 (14.9)	4 (17.4)	0.12	5 (9.1)	4 (16.7)	12 (15.8)	2 (9.1)	0.604
Superimposed infection on HBV	4 (8.9)	2 (6.7)	8 (9.2)	2 (8.7)	0.98	7 (12.7)	2 (8.3)	5 (6.6)	2 (9.1)	0.686
Hepatotoxic drugs	0 (0.0)	2 (6.7)	6 (6.9)	1 (4.3)	0.348	1 (1.8)	1 (4.2)	6 (7.9)	1 (4.5)	0.473
Active alcohol intaking	5 (11.1)	1 (3.3)	5 (5.7)	2 (8.7)	0.554	7 (12.7)	1 (4.2)	5 (6.6)	0 (0.0)	0.212
Bacterial infection	17 (37.8)	18 (60.0)	42 (48.3)	15 (65.2)	0.106	23 (41.8)	13 (54.2)	41 (53.9)	11 (50.0)	0.55
Variceal bleeding	7 (15.6)	0 (0.0)	2 (2.3)	0 (0.0)	0.002	7 (12.7)	0 (0.0)	2 (2.6)	0 (0.0)	0.018
Portal vein thrombosis	1 (2.2)	0 (0.0)	2 (2.3)	0 (0.0)	0.747	2 (3.6)	0 (0.0)	0 (0.0)	1 (4.5)	0.257
Surgery	0(0.0)	0(0.0)	1(0.7)	0(0.0)	0.767	0(0.0)	0(0.0)	1(2.7)	0(0.0)	0.093

Table S7. Prevalence of organ failure at diagnosis time point in pre-ACLF patient.

Time point	Day 4	Day 7	Day14	Day21	Day28
New onset pre-ACLF, n	28	24	20	12	10
Renal failure, n (%)	1 (3.6)	1 (4.2)	2 (10.0)	1 (5.3)	4 (20.0)
Liver failure, n (%)	24 (85.7)	23 (86.8)	19 (95.0)	9 (75.0)	6 (80.0)
Coagulation failure, n (%)	22 (78.6)	20 (83.8)	18 (90.0%)	5 (41.7)	7 (80.0)
Brain failure, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	1 (20.0)
Lung failure, n (%)	1 (3.6)	1 (4.2)	1 (5.0)	2 (16.7)	2 (20.0)
Circulation failure, n (%)	0 (0.0)	2 (8.3)	0 (0.0)	2 (16.7)	2 (13.3)

Table S8. Univariate analysis for ACLF development in HBV-related acutely decompensated cirrhotic patients in the derivation cohort.

Variables	HR	95%CI	P value
Demographic data			
Sex	1.02	0.62-1.69	0.931
ln (Age)	0.98	0.41-2.38	0.968
Acute decompensation			
AD number			
1	1	reference	
2	4.34	2.3-6.98	<0.001
≥3	12.94	8.19-23.99	<0.001
Ascites	2.01	1.19-3.39	0.009
Precipitation events			
Hepatitis B flare with HBV reactivation	4.51	2.7-7.55	<0.001
Spontaneous hepatitis B flare with high HBV-DNA load	2.56	1.43-4.6	0.002
Superimposed infection on HBV	3.20	1.48-6.91	0.003
Hepatotoxic drugs	2.17	1.05-4.47	0.036
Active alcohol intaking	1.20	0.49-2.96	0.691
Infection	3.94	2.62-5.93	<0.001
Variceal bleeding	0.37	0.18-0.77	0.008
Portal vein thrombosis	0.59	0.24-1.44	0.244
Surgery	1.17	0.29-4.76	0.824
Measurements estimating organ function			
ln (TB)	3.16	2.48-4.03	<0.001
ln (INR)	83.82	38.77-181.22	<0.001
ln (Cr)	0.86	0.45-1.63	0.639
ln (BUN)	0.94	0.61-1.43	0.76
ln (Sodium)	0.00	0-0.01	<0.001
ln (Albumin)	1.04	0.4-2.73	0.930
ln (Hemoglobin)	2.99	1.25-7.18	0.014
ln (PLT)	1.15	0.84-1.59	0.377
Systemic inflammatory parameter			
ln (WBC)	2.66	1.87-3.78	<0.001
ln (neutrophil count)	2.33	1.76-3.1	<0.001
ln (lymphocyte count)	1.04	0.74-1.46	0.814
ln (NL ratio)	1.93	1.5-2.48	<0.001

Note: The hazard ratios were obtained using the Cox Proportional-Hazards Model.

Abbreviations: HR, hazard ratio; CI, confidence interval; TB, total bilirubin; INR, International normalized ratio; WBC White blood cell count; NL ratio, neutrophil-lymphocyte ratio;

Table S9. Interaction between bacterial infections and HBV-specific hepatic precipitations in driving ACLF occurrence.

Subgroup	No. of ACLF development / Total	No. of bacterial infection (%)	Unadjusted		Adjusted	
			HR (95% CI)	p for interaction	HR (95% CI)	p for interaction
Hepatitis B flare with HBV reactivation				0.529		0.763
no	76/915	243 (26.6%)	3.36 (2.32-4.87)		2.07 (1.40-3.06)	
yes	18/55	19 (34.5%)	4.64 (2.11-10.23)		2.94 (1.18-7.32)	
Spontaneous hepatitis B flare with high HBV-DNA load				0.070		0.188
no	81/909	242 (26.6%)	2.96 (2.07-4.25)		1.88 (1.28-2.75)	
yes	13/60	20 (33.3%)	8.56 (2.81-26.11)		4.44 (1.4-14.07)	
Superimposed infection on HBV				0.167		0.619
no	87/943	256 (27.1%)	3.71 (2.62-5.25)		2.19 (1.52-3.15)	
yes	7/27	6 (22.2%)	1.21 (0.25-5.82)		0.37 (0.04-3.55)	

Statistical analysis was performed by Cox Proportional-Hazards Model.

Table S10. The competing risk regression for development of ACLF in HBV-related acutely decompensated cirrhotic patients in derivation cohort.

Characteristics	sHR* (95% CI)	p value
Demographic data		
ln (Age)	2.69 (0.82- 5.80)	0.059
Precipitant events		
Hepatitis B flare with HBV reactivation	2.39 (1.27-4.49)	0.007
Spontaneous hepatitis B flare with high HBV-DNA load	2.03 (1.14-3.62)	0.016
Superimposed infection on HBV	3.39 (1.62-7.12)	0.001
Hepatotoxic drugs	1.45 (0.59-3.56)	0.420
Bacterial infection	3.29 (1.29-8.38)	0.013
Variceal bleeding	1.94 (0.79-4.79)	0.150
Disease severity parameter		
ln (TB)	2.70 (1.76-4.15)	<0.001
ln (INR)	32.37 (12.53-83.65)	<0.001
ln (Hemoglobin)	0.78 (0.19-3.15)	0.730
ln (Sodium)	0.47 (0-238.96)	0.630
Ascites	2.18 (0.80-5.97)	0.130
AD number	0.6 (0.24-1.48)	0.260
Systemic inflammatory		
ln (WBC)	0.73 (0.45-1.19)	0.210
ln (NL ratio)	1.64 (1.2-2.25)	0.002

Note: *considering liver transplantation as competing risk

Abbreviations: TB, total bilirubin; INR, International normalized ratio; WBC White blood cell count; NL ratio, neutrophil-lymphocyte ratio;

Table S11. Multivariable logistic regression modeling to predict AD-ACLF progression in the derivation cohort.

Multivariable logistic regression modeling

Predictors	Estimate	Odds Ratio	95% CI	P value
Hepatitis B flare with HBV reactivation	1.38	3.98	1.8-8.72	0.001
Spontaneous hepatitis B flare with high HBV-DNA load	0.74	2.10	0.94-4.50	0.062
Superimposed infection on HBV	1.50	4.47	1.38-13.33	0.009
Bacterial infection	0.91	2.49	1.46-4.28	0.001
ln (TB)	0.81	2.26	1.66-3.15	<0.001
ln (INR)	4.17	64.85	19.42-235.70	<0.001
ln (NL ratio)	0.63	1.87	1.28-2.77	0.001
Intercept	-7.71	-	-	<0.001

Statistical analysis was performed by a multivariable logistic regression model.

Abbreviations: CI, confidence interval; TB, total bilirubin; INR, International normalized ratio; NL ratio, neutrophil-lymphocyte ratio;

Table S12. Baseline characteristics during hospitalization and prognosis in HBV-related acutely decompensated cirrhotic patients in derivation cohort and validation cohort

	ACLF (n= 111)	Pre-ACLF (n= 43)	UDC (n= 129)	SDC (n= 221)	p
Age (median [IQR])	49.00 [41.00, 55.50]	51.00 [46.00, 55.50]	53.00 [45.00, 60.00]	50.00 [42.00, 58.00]	0.11
Male, n (%)	99 (89.2)	37 (86.0)	87 (67.4)	180 (81.4)	<0.001
Previous decompensation (%)	40 (36.0)	9 (20.9)	72 (55.8)	81 (36.7)	<0.001
Decompensation, n (%)					
HE					<0.001
Grade 0	79 (71.2)	41 (95.3)	118 (91.5)	208 (94.1)	
Grade 1	9 (8.1)	1 (2.3)	7 (5.4)	6 (2.7)	
Grade 2	15 (13.5)	0 (0.0)	4 (3.1)	7 (3.2)	
Grade 3	4 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	
Grade 4	4 (3.6)	1 (2.3)	0 (0.0)	0 (0.0)	
Bacterial infection	29 (26.1)	13 (30.2)	17 (13.2)	43 (19.5)	0.028
Variceal bleeding	11 (9.9)	3 (7.0)	47 (36.4)	57 (25.8)	<0.001
Ascites	73 (65.8)	22 (51.2)	81 (62.8)	125 (56.6)	0.22
Laboratory data, median (IQRs)					
TB (mg/dL)	21.76 [15.57, 29.56]	21.30 [13.95, 27.70]	2.51 [1.12, 8.06]	3.22 [1.46, 10.02]	<0.001
INR	2.85 [2.27, 3.49]	1.82 [1.57, 2.06]	1.50 [1.31, 1.80]	1.41 [1.25, 1.67]	<0.001
Cr (mg/dL)	1.00 [0.74, 1.50]	0.91 [0.70, 1.03]	0.77 [0.67, 0.91]	0.77 [0.66, 0.92]	<0.001
BUN (mEq/L)	6.30 [3.72, 10.79]	4.84 [3.40, 6.65]	5.34 [4.02, 7.90]	4.80 [3.99, 6.90]	0.014
ALT (U/L)	122.00 [50.10, 559.50]	161.60 [57.75, 367.55]	32.20 [20.50, 87.70]	52.10 [25.00, 155.00]	<0.001
AST (U/L)	143.30 [73.15, 328.05]	128.50 [78.20, 374.30]	47.80 [29.00, 92.30]	66.40 [34.00, 169.00]	<0.001
Hemoglobin (g/L)	113.00 [95.00, 129.50]	119.00 [96.50, 132.00]	103.50 [80.55, 125.25]	115.00 [94.00, 129.00]	0.011
WBC (10^9/L)	6.81 [4.49, 9.40]	5.84 [4.57, 7.85]	4.09 [3.00, 6.53]	4.50 [3.16, 5.95]	<0.001
PLT (10^9/L)	80.00 [51.00, 119.00]	92.00 [61.00, 119.50]	65.50 [41.30, 102.25]	76.00 [52.00, 113.00]	0.084
NL ratio	4.69 [3.35, 8.59]	4.87 [3.04, 7.51]	3.17 [2.21, 5.38]	2.94 [1.82, 4.58]	<0.001
Sodium (mEq/L)	136.00 [131.00, 138.85]	137.30 [133.90, 139.70]	139.00 [136.00, 141.10]	138.80 [136.20, 140.60]	<0.001
Albumin (g/L)	30.70 [27.05, 34.15]	30.60 [27.50, 34.20]	31.00 [27.50, 34.00]	31.00 [26.90, 34.50]	0.976
Prealbumin, mg/L	36.00 [20.00, 52.00]	39.70 [8.82, 56.75]	53.35 [15.75, 94.05]	45.00 [3.90, 83.00]	0.419
C-reaction protein, mg/L	10.84 [6.65, 16.61]	14.40 [7.76, 19.78]	5.90 [1.70, 12.53]	7.54 [3.13, 14.05]	<0.001
Procalcitonin (ng/ml)	0.54 [0.35, 0.97]	0.50 [0.22, 0.81]	0.16 [0.09, 0.40]	0.25 [0.13, 0.60]	<0.001
HBV parameters					
HBV-DNA, log10 IU/mL	2.94 [2.00, 4.17]	2.75 [2.00, 5.22]	2.39 [2.00, 4.15]	2.64 [1.68, 4.14]	0.308
Antiviral treatment history, n (%)					0.113

antiviral naïve	65 (58.6)	21 (48.8)	53 (41.1)	103 (46.6)	
<6 months	8 (7.2)	3 (7.0)	12 (9.3)	11 (5.0)	
>6months	38 (34.2)	19 (44.2)	64 (49.6)	107 (48.4)	
Severity scores, (median [IQR])					
MELD	31.00 [27.50, 34.00]	24.00 [21.50, 26.50]	15.00 [11.00, 21.00]	15.00 [11.00, 20.00]	<0.001
iMELD	50.00 [44.50, 56.00]	44.00 [40.00, 47.50]	33.00 [27.25, 41.00]	33.00 [29.00, 39.00]	<0.001
MELD-sodium	32.00 [28.50, 35.00]	25.00 [23.00, 28.50]	15.00 [11.00, 23.00]	16.00 [12.00, 22.00]	<0.001
Child-Pugh	11.00 [10.00, 12.00]	10.00 [9.00, 11.00]	9.00 [8.00, 10.00]	9.00 [8.00, 10.00]	<0.001
CLIF-C AD	61.42 [54.80, 68.39]	52.86 [48.25, 57.27]	45.81 [38.97, 51.22]	43.17 [38.51, 48.05]	<0.001
CLIF-C ACLF	44.23 [40.15, 48.46]	38.78 [36.28, 43.20]	32.40 [27.19, 37.89]	31.62 [28.13, 35.38]	<0.001
CLIF SOFA	8.00 [7.00, 9.00]	7.00 [7.00, 7.00]	4.50 [3.00, 6.00]	5.00 [3.00, 6.00]	<0.001
CLIF OF	10.00 [10.00, 10.00]	8.00 [8.00, 9.00]	6.00 [6.00, 8.00]	7.00 [6.00, 8.00]	<0.001
Organ failure, n (%)					
Liver	92 (82.9)	35 (81.4)	26 (20.2)	46 (20.8)	<0.001
Coagulation	79 (71.2)	1 (2.3)	2 (1.6)	1 (0.5)	<0.001
Kidney	22 (19.8)	0 (0.0)	0 (0.0)	0 (0.0)	<0.001
Cerebral	8 (7.2)	1 (2.3)	0 (0.0)	0 (0.0)	<0.001
Circulation	3 (2.7)	0 (0.0)	0 (0.0)	5 (2.3)	0.231
Lungs	2 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0.068
LT-free mortality (%)					
28-day	28/100 (38.0%)	14/41 (34.1%)	8/123 (6.5%)	0 (0.0)	<0.001
90-day	53/96 (55.2%)	23/40 (57.5%)	16/116 (13.8%)	0 (0.0)	<0.001
1-year	58/94 (61.7%)	25/39 (64.1%)	25/114 (21.9%)	2/220 (0.9%)	<0.001

HE, hepatic encephalopathy; TB, total bilirubin; INR, International normalized ratio; Cr, creatinine; BUN, blood urea nitrogen; ALT alanine aminotransferase; WBC White blood cell count; PLT, platelet count; NL ratio, neutrophil-lymphocyte ratio; MELD, the Model of End-Stage Liver Disease; iMELD, integrated MELD; CLIF OF Chronic Liver Failure-Organ Failure; CLIF-SOFA, Chronic Liver Failure- Sequential Organ Failure Assessment; LT, liver transplantation.

Table S13. Characteristics and outcomes of patients included in the derivation and validation groups

Characteristics	Derivation cohort (n= 970)	Validation cohort (n= 458)	P value
Male, n (%)	771 (79.5)	360 (78.6)	0.754
Age (median [IQR])	48.88 [42.65, 57.18]	50.50 [43.00, 58.00]	0.091
Previous decompensation, n (%)	383 (39.5)	192 (41.9)	0.413
AD number, n (%)			0.027
1	491 (50.6)	264 (57.6)	
2	328 (33.8)	141 (30.8)	
≥3	151 (15.6)	53 (11.6)	
Decompensation, n (%)			
HE			0.088
Grade 0	926 (95.5)	426 (93.0)	
Grade 1	18 (1.9)	19 (4.1)	
Grade 2	19 (2.0)	11 (2.4)	
Grade 3	6 (0.6)	1 (0.2)	
Grade 4	1 (0.1)	1 (0.2)	
Ascites	676 (69.7)	269 (58.7)	<0.001
Bacterial infection	262 (27.0)	90 (19.7)	0.003
Variceal bleeding	187 (19.3)	123 (26.9)	0.002
Precipitation events, n (%)			
Hepatitis B flare with HBV reactivation	55 (5.7)	28 (6.1)	0.831
Spontaneous hepatitis B flare with high HBV-DNA load	61 (6.3)	9 (2.0)	0.001
Superimposed infection on HBV	27 (2.8)	4 (0.9)	0.034
Hepatotoxic drugs	43 (4.4)	32 (7.0)	0.058
Active alcohol intaking	44 (4.5)	42 (9.2)	0.001
Portal vein thrombosis	83 (8.6)	41 (9.0)	0.883
Surgery	17 (1.8)	8 (1.7)	1.000
Laboratory data, median (IQRs)			
TB (mg/dL)	1.38 [0.44, 2.49]	1.18 [0.33, 2.55]	0.381
INR	0.42 [0.28, 0.61]	0.41 [0.24, 0.56]	0.015
Cr (mg/dL)	-0.25 [-0.42, -0.08]	-0.25 [-0.40, -0.05]	0.470
BUN (mEq/L)	1.57 [1.30, 1.90]	1.59 [1.36, 1.95]	0.036
ALT (U/L)	4.06 [3.30, 5.22]	3.77 [3.18, 4.92]	0.001
Hemoglobin (g/L)	4.72 [4.52, 4.85]	4.73 [4.47, 4.85]	0.694
WBC (10^9/L)	1.50 [1.10, 1.86]	1.50 [1.15, 1.87]	0.732
PLT (10^9/L)	4.22 [3.85, 4.63]	4.32 [3.88, 4.71]	0.047
HBV-DNA, log10IU/ml (median [IQR])	4.10 [2.57, 6.02]	2.69 [1.76, 4.37]	<0.001
NL ratio	0.90 [0.47, 1.43]	1.14 [0.73, 1.68]	<0.001
Albumin (g/L)	3.42 [3.28, 3.54]	3.43 [3.30, 3.54]	0.321
Prealbumin, mg/L	0.30 [0.12, 0.66]	0.23 [0.11, 0.60]	0.098
C-reaction protein, mg/L	8.70 [3.28, 17.60]	7.64 [3.07, 14.74]	0.202
Severity scores, (median [IQR])			
MELD	17.00 [12.00, 23.00]	16.00 [11.00, 22.00]	0.003
iMELD	36.00 [30.00, 42.00]	34.00 [29.00, 41.00]	0.008
MELD-sodium	19.00 [13.00, 24.00]	17.00 [12.00, 23.25]	0.002
Child-Pugh	9.00 [8.00, 11.00]	9.00 [8.00, 10.00]	0.008

CLIF-C AD	44.72 [39.20, 51.61]	44.97 [39.51, 50.99]	0.688
CLIF-C ACLF	31.66 [27.30, 37.29]	32.48 [28.13, 37.74]	0.151
CLIF SOFA	5.00 [3.00, 7.00]	5.00 [3.00, 7.00]	0.973
CLIF OF	7.00 [6.00, 8.00]	7.00 [6.00, 8.00]	0.717
pre-ACLF, n (%)	94 (9.7)	43 (9.4)	0.933
LT-free mortality (%)			
28-day	49/907 (5.40%)	24/435 (5.52%)	0.549
90-day	113/893 (12.65%)	41/426 (9.62%)	0.226
1-year	169/881 (19.18%)	59/418 (14.11%)	0.095

Abbreviations: HE, hepatic encephalopathy; TB, total bilirubin; INR, International normalized ratio; Cr, creatinine; BUN, blood urea nitrogen; ALT alanine aminotransferase; WBC White blood cell count; PLT, platelet count; NL ratio, neutrophil-lymphocyte ratio; MELD, the Model of End-Stage Liver Disease; iMELD, integrated MELD; CLIF OF Chronic Liver Failure-Organ Failure; CLIF-SOFA, Chronic Liver Failure- Sequential Organ Failure Assessment; LT, liver transplantation.

Table S14. The C-index of pre-ACLF prediction model to predict outcomes and readmission in validation cohort.

	Model	iMELD	MELD-sodium	CLIF-C AD	Child-Turcotte-Pugh
	C-index (95% CI)	C-index (95% CI)	C-index (95% CI)	C-index (95% CI)	C-index (95% CI)
Outcomes					
28-day mortality	0.763 (0.662-0.864)	0.772 (0.669-0.875)	0.769 (0.671-0.867)	0.789 (0.704-0.874)	0.781 (0.700-0.862)
90-day mortality	0.781 (0.709-0.854)	0.781 (0.702-0.859)	0.764 (0.683-0.846)	0.773 (0.703-0.843)	0.718 (0.646-0.790)
1-year mortality	0.702 (0.627-0.777)	0.722 (0.647-0.798)	0.694 (0.614-0.774)	0.717 (0.646-0.789)	0.653 (0.580-0.722)
Readmission	0.543 (0.496-0.589)	0.536 (0.473-0.599)	0.521 (0.458-0.583)	0.569 (0.508-0.630)	0.505 (0.455-0.565)

Annotation: prediction of outcomes including 28-day,90-day and 1-year liver transplantation mortality was analysed by cox regression; prediction of readmission was analysed by logistic regression.

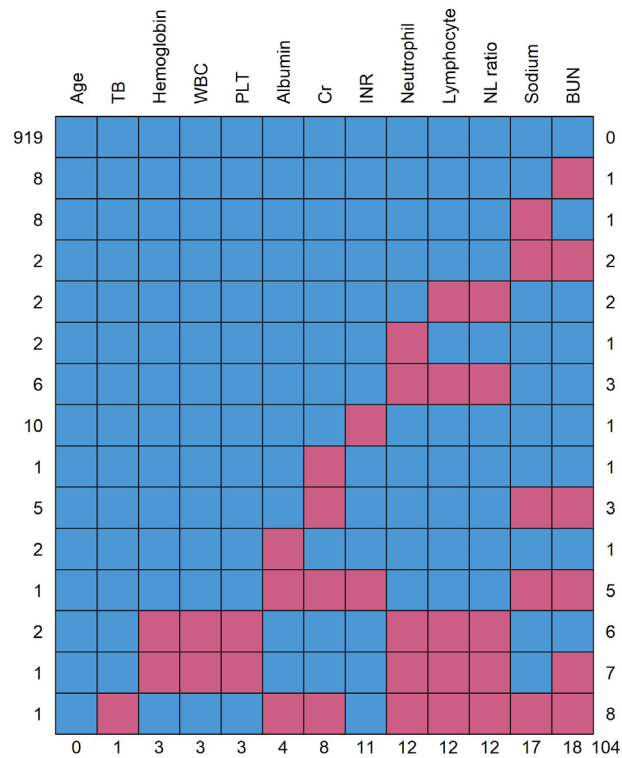**Fig. S1. Pattern of missing data of the continuous variables analyzed in the derivation cohort.**

Figure legend: Pattern of missing data of the continuous variables analyzed in the derivation cohort. The red square means data missing and the blue square means data complete. The figure shows the overall number of missing data for each variable and the distribution of different missing types. Taking the "BUN" in the rightmost column as an example, a total of 18 patients has missing data of BUN, of which 11 patients (line 2) have missing data of BUN alone, 2 patient (line 4) has missing data of BUN and sodium, 3 patients (line 10) have missing data of creatinine, sodium and BUN.

Fig. S2. Kaplan–Meier analysis of liver transplantation (LT)-free survival rates in patients with ACLF, pre-ACLF, unstable decompensated cirrhosis (UDC) and stable decompensated cirrhosis (SDC).
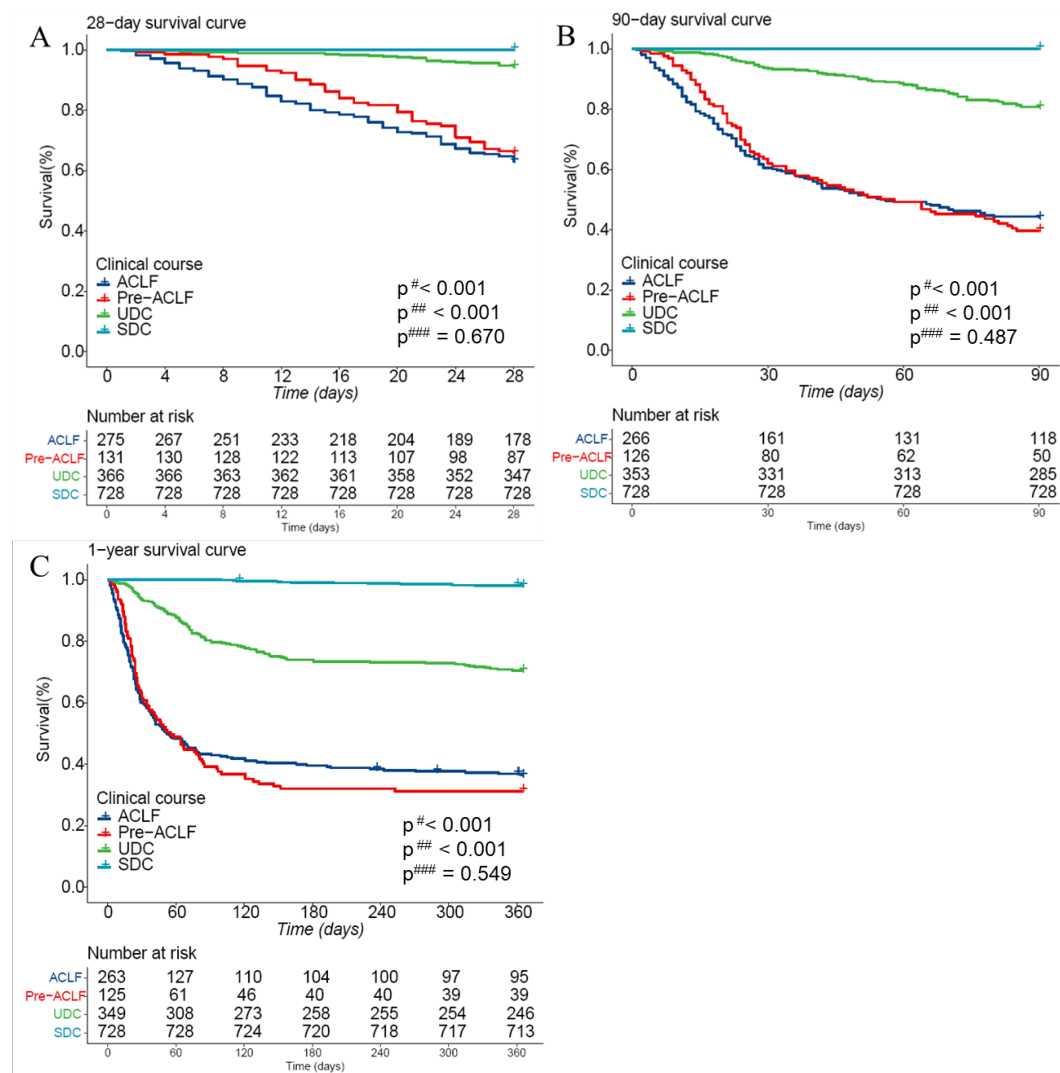
Annotation: p # denotes p value for comparisons between UDC and SDC group patients; p ## denotes p value for comparisons between UDC and pre-ACLF patients; p ### denotes p value for comparisons between ACLF and pre-ACLF.

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Fig. S3. Cumulative number of pre-ACLF at 4, 7, 14, 21, 28 days during hospitalization in the derivation cohort. (A) Fitting curve (red line) of cumulative number; (B) Bar plot of cumulative number.
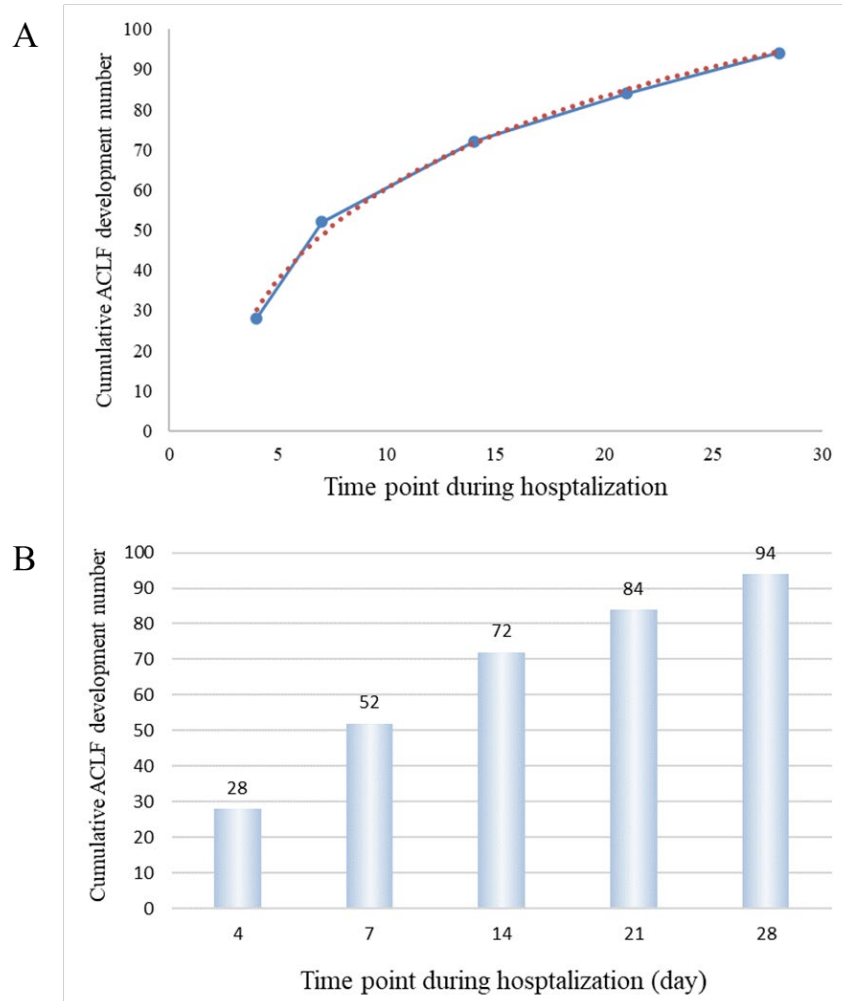
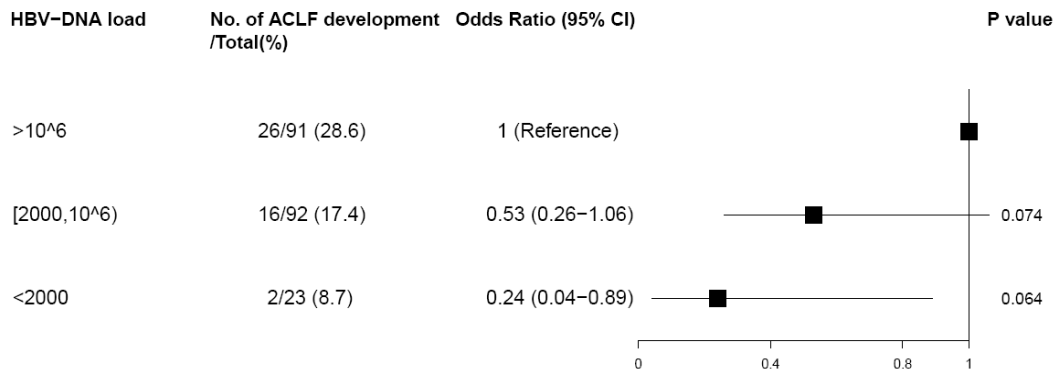**Fig. S4. Association of HBV-DNA load level on admission and ACLF development in patients with hepatitis flare.**

HBV−DNA load	No. of ACLF development /Total(%)	Odds Ratio (95% CI)		P value
>10^6	26/91 (28.6)	1 (Reference)		
[2000,10^6)	16/92 (17.4)	0.53 (0.26−1.06)		0.074
<2000	2/23 (8.7)	0.24 (0.04−0.89)		0.064

0   0.4   0.8   1

Fig. S5. Relative importance of predictors in the derivation model. (A) The deviance of predictors measured by Wald χ^2 minus the predictor degrees of freedom; (B) The percentage of relative contribution to the variance of the model.
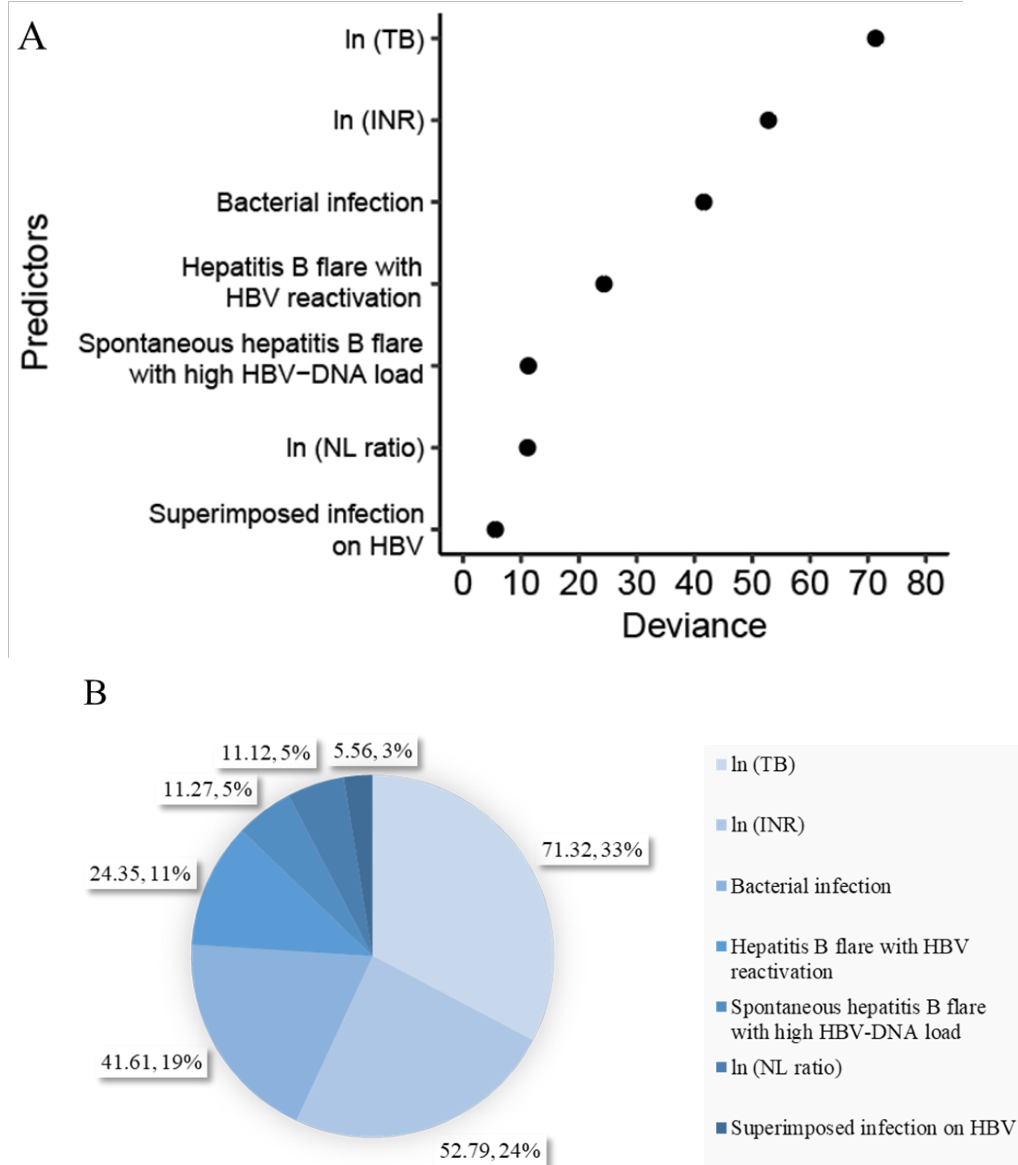
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