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Original Article

New Algorithm Rules Out Acute-on-chronic Liver Failure Development within 28 Days from Acute Decompensation of Cirrhosis

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Abstract

Background and Aims: Approximately 10% of patients with acute decompensated (AD) cirrhosis develop acute-on-chronic liver failure (ACLF) within 28 days. Such cases have high mortality and are difficult to predict. Therefore, we aimed to establish and validate an algorithm to identify these patients on hospitalization. ***Methods:*** Hospitalized patients with AD who developed ACLF within 28 days were considered pre-ACLF. Organ dysfunction was defined according to the chronic liver failure-sequential organ failure assessment (CLIF-SOFA) criteria, and proven bacterial infection was taken to indicate immune system dysfunction. A retrospective multicenter cohort and prospective one were used to derive and to validate the potential algorithm, respectively. A miss rate of <5% was acceptable for the calculating algorithm to rule out pre-ACLF. ***Results:*** In the derivation cohort (n=673), 46 patients developed ACLF within 28 days. Serum total bilirubin, creatinine, international normalized ratio, and present proven bacterial infection at admission were associated with the development of ACLF. AD patients with ≥2 organ dysfunctions had a higher risk for pre-ACLF patients [odds ratio=16.581 95% confidence interval: (4.271–64.363), p<0.001]. In the derivation cohort, 67.5% of patients (454/673) had ≤1 organ dysfunction and two patients (0.4%) were pre-ACLF, with a miss rate of 4.3% (missed/total, 2/46). In the validation cohort, 65.9% of patients (914/1388) had ≤1 organ dysfunction, and four (0.3%) of them were pre-ACLF, with a miss rate of 3.4% (missed/total, 4/117). ***Conclusions:*** AD patients with ≤1 organ dysfunction had a significantly lower risk of developing ACLF within 28 days of admission and could be safely ruled out with a pre-ACLF miss rate of <5%.

Keywords: Liver cirrhosis; Prognosis; Liver failure; End-stage liver disease; Organ dysfunction scores.

Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIF-C, Chronic Liver Failure Consortium; HBV, hepatitis B virus; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; LR−, negative likelihood.

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Introduction

Acute decompensation (AD) of cirrhosis is defined as the acute development of ascites, hepatic encephalopathy, gastrointestinal hemorrhage, bacterial infections, or any combination of these.[1] Acute-on-chronic liver failure (ACLF), the most severe phenotype of AD, is a syndrome characterized by acute clinical deterioration and is associated with organ failure and high short-term mortality.[2–6] The CANONIC study characterized ACLF and defined it as single or multiple organ failure (liver, kidney, brain, coagulation, circulation, or respiration), which is consistent with the modified sequential organ function assessment (SOFA) criteria, known as the CLIF-SOFA score.[3,7]

Patients with AD who develop ACLF in the short-term are considered as pre-ACLF. In the PREDICT study, patients with pre-ACLF were defined as those developing ACLF within 90 days after AD and were characterized by high-grade systemic inflammation at enrollment and high 90-day mortality (67%).[8] In our retrospective hepatitis B virus (HBV)-related cohort, patients who developed ACLF within 28 days after admission had high-grade of systemic inflammation and high short-term mortality that was comparable with that of ACLF patients.[9] Around 10% of AD patients developed ACLF within 28 days, 10.8% (112/1040) in the CANONIC study, 13.7% (142/1038) in the PREDICT study, and 13.3% (90/577) in our retrospective study in HBV-related AD patients.[3,8,9]

As pre-ACLF is associated with high short-term mortality, and organ function in pre-ACLF deteriorates into organ failure as per the ACLF criteria, it is urgently necessary to identify pre-ACLF prior to the development of organ failure. However, the PREDICT study, which proposed and profiled an *ex post facto* definition of pre-ACLF, did not provide a satisfactory model to distinguish patients with pre-ACLF from those with AD at their admissions.[8] As an alternative, our study aimed to establish and validate a simple algorithm to safely rule out, rather than rule in these pre-ACLF cases among patients with AD on admission.

Methods

Patients and study procedures

A retrospective cohort and a prospective multicenter cohort were used as the derivation and the validation cohorts, respectively. The derivation cohort screened all hospitalized cirrhotic patients with AD (ascites, encephalopathy, gastrointestinal hemorrhage and/or bacterial infection) between December 2011 and June 2016 at the Hepatology Unit, Nanfang Hospital. Patients were excluded if any of the following criteria were met: pregnancy, hepatocellular carcinoma, or other liver malignancies, malignancy of other organs, severe chronic extrahepatic disease, and receipt of immunosuppressive drugs. Patients within the ACLF criteria defined by EASL-CLIF were excluded from the study. For each patient, we collected the demographic data and medical history and physical examination and laboratory data at admission, during hospital stay, and at the clinic after discharge within 28 days on admission. Survival time and information related to liver transplantation and liver cancer were documented over 1 year and followed-up by telephone or until the last clinic visit.

The validation cohort, enrolled patients who were hospitalized for AD or acute liver injury at 12 teaching hospitals between January 2015 and December 2016 as previously described.[10] Patients were excluded if any of the following criteria were met: pregnancy, hepatocellular carcinoma, or other liver malignancies, malignancy of other organs, severe chronic extrahepatic disease, and receipt of immunosuppressive drugs. Patients who already had ACLF at admission according to the CANONIC study criteria, or patients without AD were also excluded from the study. During hospitalization, data were collected at 1, 4, 7, 14, 21, and 28 days, or the last day if the patient was hospitalized if less than 28 days. The patients were followed-up regularly after discharge at clinic visits or by telephone calls. Survival time and information related to liver transplantation and liver cancer were documented.

Diagnostic criteria and definition

Hepatic cirrhosis was diagnosed based on previous liver biopsy findings or a composite of clinical features, radiological evidences, and laboratory data.[11] The diagnostic criteria for AD upon hospitalization were based on the development of ascites, hepatic encephalopathy, gastrointestinal hemorrhage, infection, or any combination of these.[12–16] The presence of proven bacterial infection was defined as follows.[17–21] Spontaneous bacterial peritonitis (SBP) was defined as a polymorphonuclear cell count of ≥250/mm^3 in the ascitic fluid. Urinary tract bacterial infection was defined as urine white blood cell count of >15/high-power field with either positive urine gram stain or culture. Bacteremia was diagnosed on the basis of positive blood bacterial cultures. Pneumonia was diagnosed according to presence of clinical signs of infection and new infiltrates on chest X-ray or computerized tomography scanning. Skin and soft tissue bacterial infections were considered when clinical signs of infection were present, or with positive secretion cultures.

In our non-ACLF patients, the severity of hepatic-related organ impairments was evaluated and categorized according to the CLIF-SOFA criteria.[3,7] Liver impairment was defined as total bilirubin ≥6 mg/mL and liver failure as total bilirubin ≥12 mg/dL.[3] Coagulation impairment was defined as international normalized ratio (INR) ≥1.5, and coagulation failure as an INR ≥2.5.[3] Kidney dysfunction was defined as serum creatinine level ≥1.5 mg/dL and kidney failure as a serum creatinine level ≥2 mg/dL, consistent with the CLIF-SOFA diagnosis criteria.[3] Moreover, the presence of proven bacterial infection was considered as an immune system impairment.[5,22]

The medical interventions for ACLF were antimicrobial therapy in patients with infection,[4,5] potent antiviral drugs, such as tenofovir, or entecavir, in patients with HBV infection,[23] and supportive therapy in patients with organ failure.[4,5] Terlipressin or noradrenaline combined with volume expansion with albumin was the first-choice treatment for hepatorenal syndrome.[24] In this study, patients who developed ACLF within 28 days after admission were considered to have pre-ACLF.[9] A miss rate (missed pre-ACLF/total pre-ACLF) of <5% was acceptable for calculating the algorithm for ruling out pre-ACLF.[25]

Statistical analysis

The results were reported as count and percentage (%) for categorical variables and as median and interquartile range for continuous variables. For the univariate statistical analyses, either the Chi-square or Fisher's exact test was used to compare categorical variables, and the Mann-Whitney U test, Kruskal-Wallis test, and nonparametric analysis of variance were used to compare continuous variables. Logistic regression models were used to identify factors associated with the development of ACLF within 28 days after enrollment. Factors with clinically and statistically significant associations

(p<0.05) with the development of ACLF in the univariate analyses were entered into multivariate analysis. In order to rule out ACLF development within 28 days, the value corresponding to the negative likelihood (LR−) closest to 0.05 was chosen for the cutoffs of the number of dysfunctional organs, Model for End-Stage Liver Disease (MELD), MELD-sodium (MELD-Na), and the CLIF consortium AD scores.[25] The cutoffs were chosen in the derivation cohort in order to achieve low LR− values and were validated in the validation cohort. Mortality rates were estimated as transplant-free mortality. To compare the predictive capability of different prognostic scoring systems, areas under the receiver operating curve were calculated and compared using the Z-test. Kaplan-Meier analysis was then used to compare mortality rates between the different groups. The significance level was set at p<0.05. Statistical analysis was performed using SPSS (version 22.0; IBM Corp., Armonk, NY, USA) and GraphPad Prism (version 8; GraphPad Software, California, USA).

Results

Patient enrollment and ACLF development within 28 days

In the retrospective cohort, 781 AD patients were screened, and 108 were diagnosed with ACLF at admission were excluded. A total of 673 non-ACLF patients were included in the current analysis as the derivation cohort (Fig. 1A). Forty-six (6.8%) patients developed ACLF within 28 days (pre-ACLF) after admission. In the prospective multicenter cohort, 2600 patients with AD or acute liver injury were screened. Overall, 999 patients without AD were excluded from the analysis, and 213 patients with ACLF at admission were also excluded. The remaining 1388 AD patients were included in the study as the validation cohort. In that cohort, 117 patients developed ACLF within 28 days after admission (Fig. 1B).

Baseline characteristics of patients with ACLF development within 28 days

Chronic HBV infection was the predominant etiologic factor for chronic liver disease across the two cohorts (Table 1). Infections as decompensation events were more frequent (derivation cohort, 58.7% vs. 25.7%, p<0.001; validation cohort, 47.9% vs. 21.1%, p<0.001) in patients encountering ACLF development than in those without ACLF development. Pneumonia and SBP were common types of infection in patients who developed into ACLF within 28 days after admission. White blood cell count, neutrophil percentage, and C-reactive protein levels were significantly higher in patients who developed ACLF than in those without ACLF development within 28 days (Table 1). There was no significant difference in the occurrence of hepatic encephalopathy between patients with ACLF development and those without ACLF development (derivation cohort, 6.5% vs. 5.6%, p=0.79; validation cohort, 2.6% vs. 5.3%, p=0.2; Table 1). In both cohorts, pre-ACLF patients had higher levels of total bilirubin and serum creatinine and INR than the AD group at enrollment (Table 1). Moreover, the commonly adopted liver diseases severity scores, including the Chronic Liver Failure Consortium (CLIF-C) AD, MELD, and MELD-Na, were significantly higher in the pre-ACLF group than in those without ACLF development (Table 1). Patients with pre-ACLF had a higher 28-day mortality than those without ACLF development (derivation cohort, 41.3% vs. 2.7%, p<0.001; validation cohort, 27.0% vs. 2.1%, p<0.001; Table 1). The organ failures were shown in Supplementary Table 1.

Organ impairment associated with ACLF development within 28 days in the derivation cohort

Various baseline clinical and laboratory variables at enrollment were analyzed as potential predictors of ACLF development in the univariate analysis (Table 2). Hepatic encephalopathy, gastrointestinal hemorrhage, ascites, mean arterial pressure, and age were not significantly associated with the development of ACLF in the univariate analysis (p<0.05). Serum bilirubin level, serum creatinine level, INR, alanine aminotransferase level, aspartate aminotransferase level, white blood cell count, serum C-reactive protein level, serum sodium level, and infection at enrollment were significantly associated with the development of ACLF (p<0.05) in the univariate analysis and were entered into the multivariate analysis. Only the serum bilirubin level [odds ratio (OR)=1.004; 95% confidence interval (CI), 1.002–1.006; p<0.001)], serum creatinine level (OR=1.014; 95% CI, 1.000–1.029; p=0.041), INR (OR=3.403; 95% CI, 1.745–6.638; p<0.001), and presence of proven bacterial infection (OR=2.537; 95% CI, 1.202–5.354; p=0.015) were identified as independent predictors associated with ACLF development within 28 days in the multivariate analysis.

Pre-ACLF patients had ≥2 organ dysfunctions at admission

In the present study, among non-ACLF patients, we defined liver dysfunction as total bilirubin ≥6 mg/dL, coagulation dysfunction as an INR ≥1.5, kidney dysfunction as serum creatinine 1.5–1.9 mg/dL, and immune system dysfunction as the presence of proven bacterial infection. The patients who developed ACLF within 28 days predominantly had ≥2 organ dysfunctions, both in the derivation cohort and the validation cohort (Supplementary Fig. 1). The types of organ dysfunction are shown in Supplementary Figure 2, and coagulation dysfunction alone or coexisting with liver dysfunction or with proven bacterial infections was common in both cohorts. In the derivation cohort, 95.7% pre-ACLF patients (44/46) had two organ dysfunctions (n=24) or three organ dysfunctions (n=20; Table 3). Only two (4.4%) pre-ACLF patients had fewer than two organ dysfunctions (no organ dysfunction, n=1; single organ dysfunction, n=1). In contrast, no organ dysfunction (208/627, 33.2%) and single organ dysfunction (244/627, 38.9%) were found in 72.1% of the patients without ACLF development, and two or three organ dysfunctions were found in the remaining 27.9% (175/627) of AD patients who did not develop ACLF within 28 days (Table 3).

Similar to the derivation cohort, 4/117 of patients with pre-ACLF (3.5%) had ≤1 organ dysfunction (no organ dysfunction, n=1; single organ dysfunction, n=3), and the remaining pre-ACLF patients (113/117, 96.5%) in the validation cohort had two or three organ dysfunctions (Table 3). In patients who did not develop ACLF within 28 days after admission, 72.5% had ≤1 organ dysfunction, and 27.5% of patients had two or three dysfunctional organs without development of ACLF within 28 days after admission (Table 3). None of our patients had four or more organ dysfunctions simultaneously.

In the derivation cohort, the OR value in patients with single organ dysfunction was not significantly different from that of those without organ dysfunction (p=0.91; Table 3). Patients with two organ dysfunctions had a significantly higher risk of ACLF development within 28-days than those without organ dysfunction [OR=14.986, 95% CI, (2.192–102.45); p<0.001]. Likewise, patients with three organ dysfunctions had a significantly higher risk of ACLF development within 28-days than those without organ dysfunction

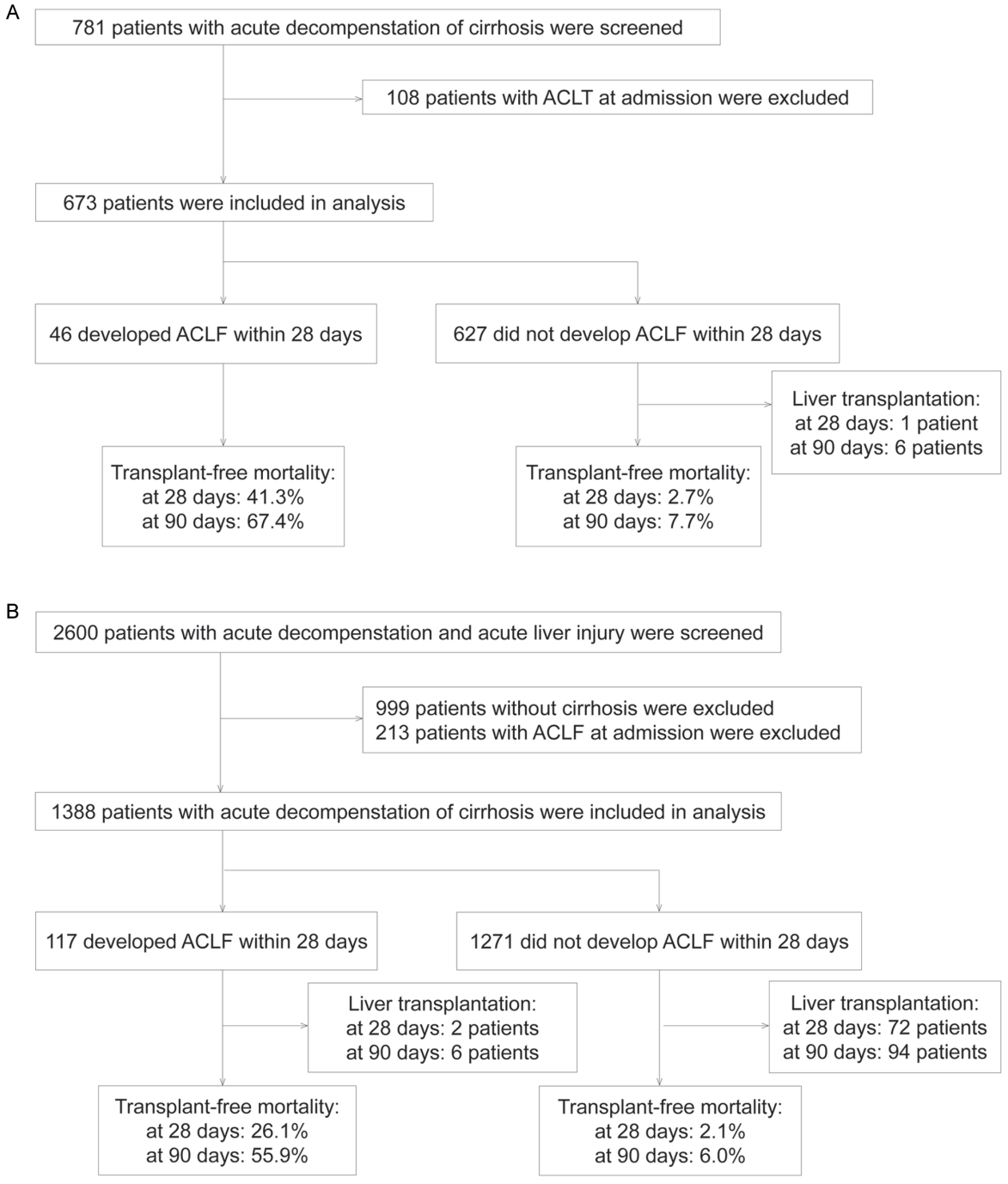

Fig. 1. Flowchart of patient selection. (A) The derivation cohort; (B) The validation cohort. ACLF, acute-on-chronic liver failure.

[OR=17.902, 95% CI (2.642–121.308), $p<0.001$; Table 3]. In the validation cohort, a greater risk for ACLF development within 28 days was also found in patients with two organ dysfunctions [OR=13.213, 95% CI, (4.371–39.942), $p<0.001$] or three organ dysfunctions [OR=15.529, 95% CI (5.164–46.692), $p<0.001$] than in patients without organ dysfunction (Table 3). Compared with that of patients with ≤1 organ dysfunction, those with ≥2 organ dysfunctions had a greater risk of developing ACLF within 28 days in both the derivation cohort [OR=16.581, 95% CI, (4.271–64.363); $p<0.001$] and validation cohort [OR=20.942, 95% CI, (7.989–54.898), $p<0.001$; Table 3).

The presence of ≤1 organ dysfunction at admission safely ruled out ACLF development within 28 days

To establish an algorithm able to safely rule out patients who would develop ACLF, ≤1 was chosen as the cutoff value for the number of dysfunctional organs, as the negative likelihood

Table 1. Baseline characteristics of patients in the derivation and validation cohorts

	Derivation cohort (*n*=673)			Validation cohort (*n*=1388)		
	ACLF development (No) (*n*=627)	**ACLF development (Yes) (*n*=46)**	***p*-value**	**ACLF development (No) (*n*=1271)**	**ACLF development (Yes) (*n*=117)**	***p*-value**
Male sex	483 (77.0)	32 (69.6)	0.249	918 (72.2)	89 (76.1)	0.373
Age in years	51 (44–59)	52 (46–59)	0.408	50 (43–59)	41 (43–60)	0.700
Etiology of cirrhosis						
Hepatitis B virus	499 (79.6)	36 (78.3)	0.83	1020 (80.3)	98 (83.8)	0.359
Alcohol	80 (12.8)	5 (10.9)	0.71	223 (17.5)	20 (17.1)	0.902
Hepatitis C virus	30 (4.8)	1 (2.2)	0.415	124 (9.8)	9 (7.7)	0.468
Others	68 (10.8)	8 (17.4)	0.176	88 (6.9)	6 (13.0)	0.460
Ascites	563 (89.8)	44 (95.7)	0.197	837 (65.9)	98 (83.8)	<0.001
Hepatic encephalopathy	35 (5.6)	3 (6.5)	0.790	67 (5.3)	3 (2.6)	0.200
Gastrointestinal bleeding	60 (9.6)	7 (15.2)	0.217	244 (19.2)	11 (9.4)	0.009
Infections	161 (25.7)	27 (58.7)	<0.001	268 (21.1)	67 (57.3)	<0.001
Pneumonia	121 (19.3)	22 (47.8)		245 (19.3)	61 (52.1)	
Spontaneous bacterial peritonitis	31 (4.9)	9 (19.6)		22 (1.7)	3 (1.7)	
Bacteremia	18 (2.9)	1 (3.2)		13 (1.0)	6 (5.1)	
Urinary tract	17 (2.7)	1 (3.7)		9 (0.7)	0	
Laboratory data						
Serum bilirubin(mg/dL)	2.3 (1.2–4.6)	12.3 (6.0–25.7)	<0.001	2.9 (1.4–7.9)	17.6 (8.8–26.6)	<0.001
Alanine aminotransferase(U/L)	40.9 (26.0–68.0)	74.5 (40.9–260.5)	<0.001	54 (26–167)	167 (60–415)	<0.001
Aspartate aminotransferase(U/L)	64.3 (41.4–110.2)	129.7 (78–331.5)	<0.001	73 (39–165)	175 (101–458)	<0.001
Serum sodium (mmol/L)	139.0 (136.3–141.0)	137.0 (133.0–138.6)	<0.001	138.4 (135.7–141.0)	136.0 (132.4–139.0)	<0.001
Platelet count (10^9/L)	77 (50–114)	80.5 (57.3–110.3)	0.754	76 (50–121)	73 (47.5–103.5)	0.301
International Normalized Ratio	1.52 (1.31–1.77)	2.13 (1.64–2.40)	<0.001	1.4 (1.2–1.6)	2.1 (1.7–2.3)	<0.001
Serum creatinine(mg/dL)	0.79 (0.66–0.94)	0.87 (0.71–1.22)	0.01	0.75 (0.62–0.89)	0.74 (0.57–0.93)	0.076
White-cell count(10^9/L)	4.6 (3.1–6.6)	7.2 (5.0–11.0)	<0.001	4.35 (2.98–6.26)	5.8 (4.2–7.5)	<0.001
Neutrophil(N%)	60.4 (51.7–69.6)	73.6 (66.8–80.0)	<0.001	62.9 (53.2–71.5)	70.6 (61.1–77.2)	<0.001
Serum C-reactive protein(mg/L)	6.6 (2.5–15.5)	17.0 (9.3–29.3)	<0.001	7.27 (2.84–15.4)	15.4 (8.4–22.9)	<0.001
MELD	14.9±5.8	23.4±5.5	<0.001	15.5±5.6	24.4±4.3	<0.001
MELD-sodium	18.2±8.5	30.8±9.6	<0.001	18.4±12.0	26.3±4.4	<0.001
CLIF-C AD	45.3±8.2	56.4±7.8	<0.001	44.4±11.5	51.7±9.1	<0.001
Transplant-free mortality						
28-day	17 (2.7)	19 (41.3)	<0.001	25 (2.1)	31 (27.0)	<0.001
90-day	48 (7.7)	31 (67.4)	<0.001	71 (6.0)	63 (56.8)	<0.001
180-day	62 (9.9)	33 (71.7)	<0.001	108 (9.2)	73 (65.8)	<0.001
1-year	80 (12.8)	33 (71.7)	<0.001	156 (13.3)	75 (67.6)	<0.001

Qualitative data are numbers and percentages (%); quantitative data are medians (25–75% quantiles), or means and standard deviation. ACLF, acute-on chronic liver failure; CLIF-C AD, CLIF Consortium acute decompensation; MAP, mean arterial pressure; MELD, model for end-stage liver disease.

Table 2. Baseline variables associated with ACLF development within 28 days in the derivation cohort

	Univariate analysis			Multivariate analysis		
	OR estimate	**95% CI**	***p*-value**	**OR estimate**	**95% CI**	***p*-value**
Serum bilirubin	1.006	1.004–1.007	<0.001	1.004	1.002–1.006	<0.001
Serum creatinine	1.022	1.01–1.034	<0.001	1.014	1.000–1.029	0.041
International Normalized Ratio	6.625	3.531–12.43	<0.001	3.403	1.745–6.638	<0.001
Alanine aminotransferase	1.002	1.001–1.003	<0.001	1.001	0.997–1.003	0.894
Aspartate aminotransferase	1.002	1.001–1.002	<0.001	1.001	0.999–1.004	0.268
White-cell count	1.149	1.089–1.213	<0.001	1.041	0.974–1.112	0.234
Serum C-reactive protein	1.01	1.002–1.018	0.017	0.999	0.986–1.011	0.833
Serum sodium	0.892	0.842–0.945	<0.001	0.953	0.886–1.026	0.200
Infection at enrollment	3.986	2.15–7.389	<0.001	2.537	1.202–5.354	0.015
Hepatic encephalopathy	1.18	0.349–3.993	0.79	–	–	–
Gastrointestinal hemorrhage	1.696	0.727–3.958	0.222	–	–	–
Ascites	2.501	0.592–10.56	0.212	–	–	–
Mean arterial pressure	0.688	0.155–3.047	0.622	–	–	–
Age	1.012	0.985–1.04	0.375	–	–	–

ACLF, acute-on chronic liver failure; CI, confidence interval; OR, odds ratio.

ratio (LR−) was closest to 0.05 in both the derivation cohort (LR−=0.06) and the validation cohort (LR−=0.05. Table 4). In the derivation cohort, two patients with ≤1 organ dysfunction developed ACLF within 28 days, with a pre-ACLF miss rate (missed ACLF development/total ACLF development) of 4.3% (2/46). Four patients with ≤1 organ dysfunction at admission developed ACLF in the validation cohort, with a miss rate of 3.4% (4/117). The presence of ≤1 organ dysfunction in ruling out ACLF development within 28 days was tested in the derivation and the validation cohorts (Table 4). The algorithm achieved high sensitivity (95.7%, derivation cohort; 96.6%, validation cohort) and negative predictive value (both 99.6% in the derivation and validation cohorts; Table 4). In the derivation cohort, 67.5% (454/673) of patients had ≤1 organ dysfunction, and 0.4% (2/454) of those patients developed ACLF within 28 days (Fig. 2A). In contrast, 32.5% of patients (219/673) had ≥2 organ dysfunctions, and 20.1% of those patients (44/219) developed ACLF within 28 days after admission (Fig. 2A). In the validation cohort, the proportion of patients with ≤1 organ dysfunction was 65.9% (914/1388) and of those, 0.4% (4/914) developed ACLF within 28 days after admission (Fig. 2B). In patients with ≥2 organ dysfunctions, 23.8% developed ACLF within 28 days after admission (Fig. 2B). Patients with ≥2 organ dysfunctions had a greater 4-week mortality than those with ≤1 organ dysfunction, in both the derivation cohort (12.4% vs. 2%, $p<0.001$; Fig. 3A) and the validation cohort (9.5% vs. 1.5%, $p<0.001$; Fig. 3B). Both the 12-week and 1-year mortality rates were higher in patients with ≥2 organ dysfunctions than in those with ≤1 organ dysfunction (Fig. 3A, B).

The cutoffs of commonly acknowledged liver disease severity scores, such as the MELD, MELD-Na, and CLIF-C AD

Table 3. Number of organ dysfunctions at admission associated with ACLF development within 28 days

Number of organ dysfunctions, *n* (%)	**Derivation cohort (*n*=673)**				**Validation cohort (*n*=1388)**			
	ACLF development, (No) (*n*=627)	**ACLF development (Yes) (*n*=46)**	**OR (95% CI)**	***p*-value**	**ACLF development (No) (*n*=1271)**	**ACLF development (Yes) (*n*=117)**	**OR (95% CI)**	***p*-value**
None	208 (33.2)	1 (2.2)	1.0		454 (35.7)	3 (2.6)	1.0	
Single	244 (38.9)	1 (2.2)	0.920 (0.229–3.693)	0.910[a]	456 (35.9)	1 (0.9)	0.665 (0.376–1.176)	0.316[a]
Two	139 (22.2)	24 (52.2)	14.986 (2.192–102.45)	<0.001[a]	279 (22.0)	61 (52.1)	13.213 (4.371–39.942)	<0.001[a]
Three or more	36 (5.7)	20 (43.5)	17.902 (2.642–121.308)	<0.001[a]	82 (6.5)	52 (44.4)	15.529 (5.164–46.692)	<0.001[a]
≤1 organ	452 (72.1)	2 (4.3)	1.0		910 (71.6)	4 (3.4)	1.0	
≥2 organ	175 (27.9)	44 (95.7)	16.581 (4.271–64.363)	<0.001[b]	361 (28.4)	113 (96.6)	20.942 (7.989–54.898)	<0.001[b]

[a]Compared with none organ dysfunction; [b]Compared with ≤1 organ dysfunctions. ACLF, acute-on chronic liver failure; CI, confidence interval; OR, odds ratio.

Table 4. Performance of ≤1 organ dysfunction at admission in ruling out patients with ACLF development within 28 days

	Derivation cohort (*n*=673)	Validation cohort (*n*=1388)
True positive, *n*	44	113
False positive, *n*	175	361
True negative, *n*	452	910
False negative, *n*	2	4
Sensitivity (%)	95.7	96.6
Specificity (%)	72.1	71.6
Positive predictive value (%)	20.1	23.8
Negative predictive value (%)	99.6	99.6
Positive likelihood ratio	3.43	3.40
Negative likelihood ratio	0.06	0.05
Pre-ACLF miss rate% (missed/total)	4.3 (2/46)	3.4 (4/117)
Rate of ruling out patients (%)	67.5	65.9

ACLF, acute-on chronic liver failure.

scores, were set at 12, 15, and 42 respectively, when the LR− value was closest to 0.05 in the derivation cohort. A MELD score of <12 was able to rule out patients with pre-ACLF safely, with a miss rate of <5% (2.2% in the derivation cohort and 0.9% in the validation cohort, Supplementary Table 2). However, the proportion of AD patients with a MELD score <12 was significantly lower than it was in those with ≤1 organ dysfunction, in both the derivation cohort (26.0% vs. 67.5%, $p<0.001$) and in the validation cohort (23.6% vs. 65.9%, $p<0.001$). Similarly, a MELD-Na score of <15 was also able to rule out pre-ACLF safely, both in the derivation (miss rate 2.2%) and validation cohorts (miss rate 3.4%), but with a rule out capability inferior to that for those with ≤1 organ dysfunction, in both the derivation (40.1% vs. 67.5%, $p<0.001$) and the validation cohorts (38.3% vs. 65.9%, $p<0.001$). Furthermore, a CLIF-C AD score with a cutoff of 42 had an unacceptably high miss rate of 12.8% (15/117) in the validation cohort (Supplementary Table 2).

Discussion

In this study, we found that organ dysfunction including the liver, coagulation, and kidney, and proven bacterial infection (a clinical phenotype of immune system dysfunction) at admission were independently associated with ACLF development within 28 days in AD patients. In patients with ≤1 organ dysfunction at admission, ACLF development within 28 days could be safely ruled out with a miss rate (missed pre-ACLF/total pre-ACLF) of <5%. AD patients with ≤1 dysfunctional organ at admission accounted for over 60% of patients in both cohorts, and had a very low risk of ACLF development within 28 days (<0.5%). Short-term mortality in AD patients is predominantly attributed to ACLF development.[3,8] Distinguishing patients who will develop ACLF from those who will not develop ACLF, at the time of admission or as early as possible, is of great clinical significance. However, there is currently no satisfactory tool or model available to accurately identify patients at admission who may subsequently develop ACLF. One possible explanation is that the occurrence of ACLF development within 28 days was rather low in the CANONIC study (10.8%) and PREDICT study (13.7%).[3,8] The rate of ACLF development within 28 days in our AD patients was 6.8% in the derivation cohort and 8.7% in the validation cohort. Hence, the alternative option is to stratify and safely rule out patients who will not develop ACLF within a short period.

In our study, ACLF development within 28 days was independently associated with proven bacterial infection at admission. Proven bacterial infections are commonly diagnosed and considered to be the main driver of acute decompensation, and have been repeatedly found to be the leading precipitating factor for ACLF development.[3,16,26,27] The liver regulates homeostasis of the immune system and has a role in immune surveillance by defending against blood-borne pathogens via its double blood supply.[28,29] The hepatic architecture and cellular organization is disrupted, and its ability to synthesize proteins, are compromised in liver cirrhosis.[28,30] Dysfunctional immune cells in the circulation and intestine tissues have been reported in patients with liver cirrhosis.[31] Cirrhosis-associated immune dysfunction results in a poor response to bacterial challenge, with increased susceptibility to bacterial infection being accompanied by high mortality and multiorgan inflammatory damage.[16,31] In our study, similar to that in previous studies, patients who developed ACLF frequently presented SBP and pneumonia as triggering events.[20] A recent study by Wong *et al*.[20] reported that among the different types of infections, pneumonia, and SBP were independently associated with an increased risk of ACLF development.[20] Bacterial infection is considered to be a clinical phenotype of bacterial translocation and dysbiosis within the context of cirrhosis-associated immune dysfunction and is acknowledged as a hallmark of immune system dysfunction in AD patients.[18,22,31–33]

ACLF development within 28 days in our AD patients was also independently associated with levels of serum bilirubin and creatinine and INR, which reflect the three most frequent organ impairments, liver, kidney, and coagulation. AD patients with impaired organs (liver, coagulation, or kidney) and proven bacterial infection having the clinical phenotype of cirrhosis-associated immune dysfunction were supposed to have an increased risk of ACLF development afterwards. Herein, and consistent with the CLIF-SOFA definition, organ dysfunction was defined as total bilirubin ≥6 mg/dL for liver dysfunction, an INR ≥1.5 for coagulation dysfunction, serum creatinine 1.5–2.0 mg/dL for kidney dysfunction, and presence of proven bacterial infection for cirrhosis-associated immune dysfunction. We found that AD patients with ≥2 dysfunctional organs had a greater risk for ACLF development within 28 days than those with ≤1 dysfunctional organ in both cohorts. With an LR− value closest to 0.05 in both of our cohorts, a value of ≤1 organ dysfunction was chosen

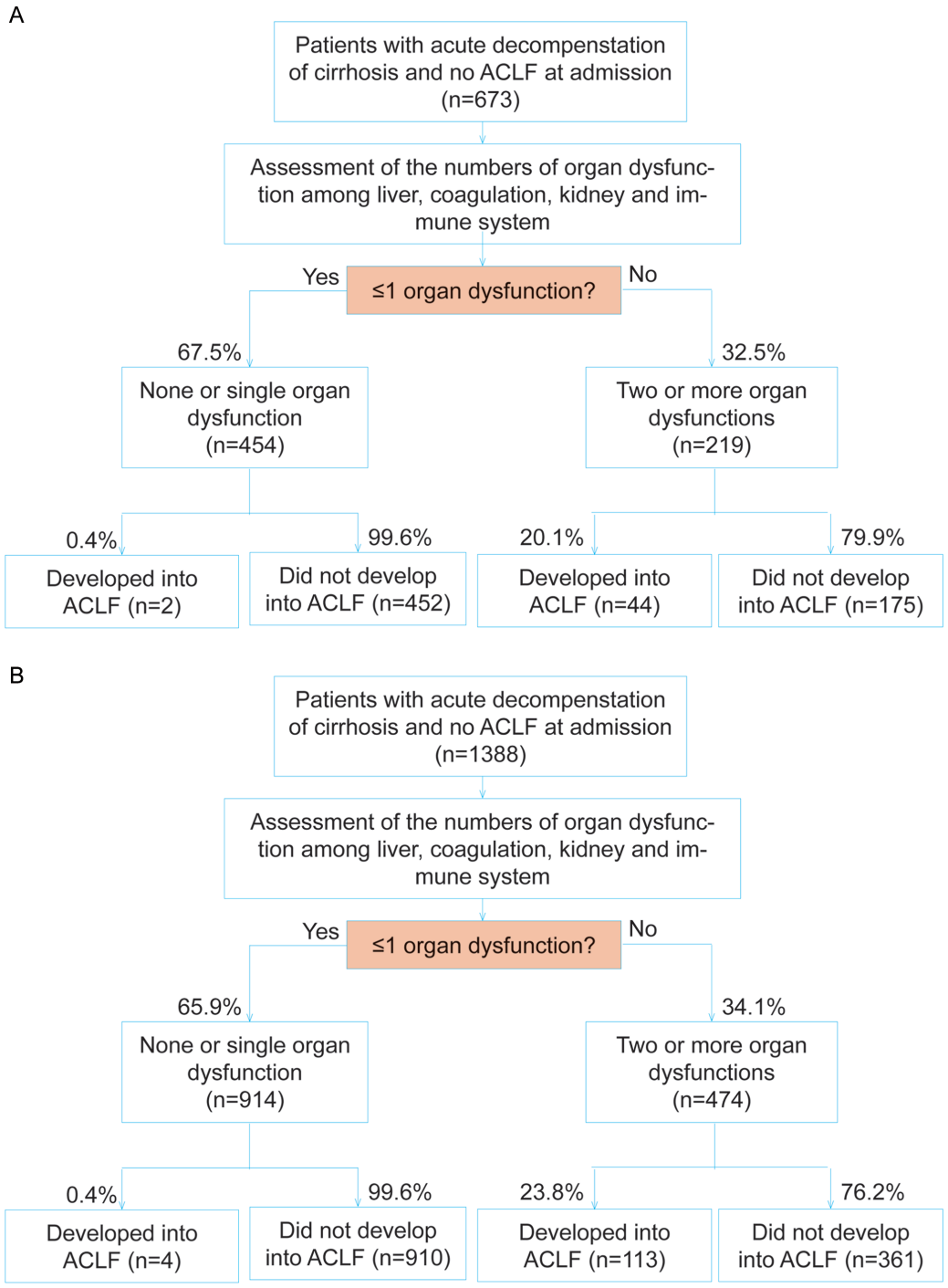

Fig. 2. Number of organ dysfunctions (≤1) at admission in acute decompensated cirrhosis patients safely ruled out ACLF development within 28 days. (A) Derivation cohort; (B) Validation cohort. ACLF, acute-on-chronic liver failure.

and tested for its ability to rule out ACLF development within 28 days in our patients. This simple algorithm (≤1 organ dysfunction) achieved acceptable miss rates (i.e. missed ACLF development/total ACLF development), as the values were both <5%, 4.3% in the derivation cohort and 3.4% in the validation cohort. Comparing commonly acknowledged liver severity scores with this simple algorithm (≤1 organ dysfunction), MELD score and MELD-Na score were shown to be inferior in ruling out pre-ACLF patients, both in the derivation cohort and in the validation cohort. Similarly, the CLIF-C AD score did not have consistent performance in our validation cohort, as the pre-ACLF miss rate was >5%.

Approximately two-thirds (67.5% in the derivation cohort and 65.9% in the validation cohort) of AD patients had ≤1 organ dysfunction and a low probability (both <0.5% in the derivation and validation cohorts) of developing ACLF within

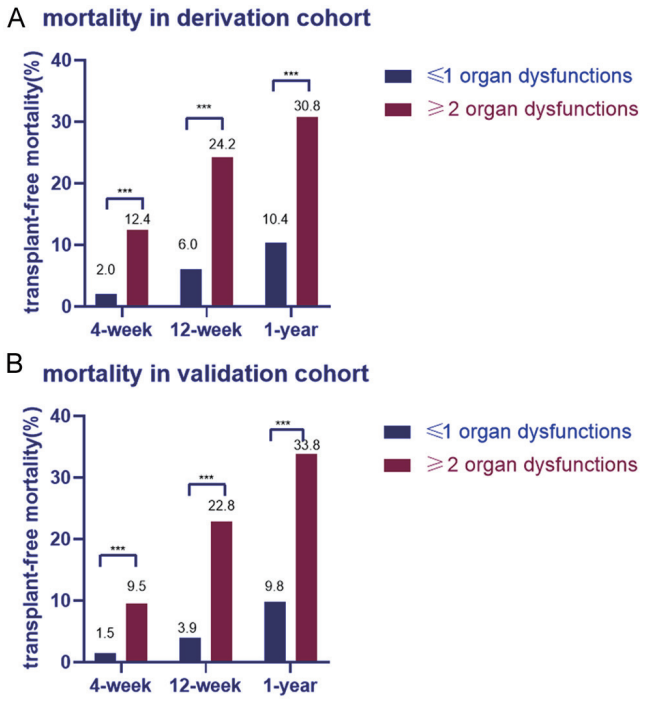

Fig. 3. Mortality of patients with ≤1 organ dysfunctions and of those with ≥2 organ dysfunctions. (A) Derivation cohort; (B) Validation cohort. ***p<0.001.

28 days after admission. Those patients consistently had very low 12-week and 1-year mortality rates. The findings indicated that AD liver cirrhosis patients with ≤1 organ dysfunction were at a low risk of disease progression and mortality within 28 days and even longer. From the perspective of patient stratification, AD patients with ≤1 organ dysfunction at admission were not an appropriate target population for surveillance of ACLF development either during the hospital stay or within 28 days afterward.

In contrast, AD patients with ≥2 dysfunctional organs had a higher risk of developing ACLF within 28 days (20.1% in the derivation cohort and 23.8% in the validation cohort). Our data suggest that such patients should be prioritized for ACLF development surveillance during their hospital stay. However, the currently acknowledged models, including MELD, MELD-Na, CLIF-C AD, or CLIF-C ACLF-D, were not satisfactory for identifying ACLF development at admission even in those with ≥2 organ dysfunctions (Supplementary Fig. 3). More data, including the discovery of novel biomarkers for highly accurate prediction of ACLF development, are needed in the future. More intensive strategies are warranted to screen and prevent ACLF development in AD patients with ≥2 organ dysfunctions.

Our study has limitations. First, in both the derivation and the validation cohorts, the clinical and laboratory data of patients were recorded only during the period of hospitalization from the time of enrollment to a maximum of 28 days, so there were no data on ACLF development within 90 days after admission. As the PREDICT study has indicated that approximately 45% of ACLF development occurred after 28 days and within 90 days, the performance of this simple algorithm (≤1 organ dysfunction) in ruling out ACLF development within 90 days needs further validation in a proper cohort and in future prospective investigations. Second, patients with HBV-related liver cirrhosis constituted the majority of our cases, and they had different characteristics in terms of organ impairments compared with other cohorts of predominantly alcoholic hepatitis-related AD patients.[3,8,26] The types of organ dysfunction associated with ACLF development in other cohorts may not be identical and warrants further investigation. Third, we did not find an independent association between other organ impairments (e.g. brain, lung, or circulation) and ACLF development in our data. A possible explanation for it was that the prevalence of those organ impairments was low and did not reach statistical significance in our analysis. Fourth, the diagnosis of proven bacterial infections in clinical practice, which depends on the turnover time of traditional blood and/or urine culture in laboratories, may require a couple of days. That would delay the prompt stratification of AD patients using this simple algorithm in some scenarios, such as in the liver clinic or emergency unit. However, rapidly acquired laboratory biomarkers related to infections such as white blood cell count, neutrophil count, and serum C-reactive protein were not entered into the model. Finally, this algorithm (≤1 organ dysfunction) calculated and utilized the clinical and laboratory data of patients at admission. It would be expected that an algorithm including the dynamics of organ impairment would have a better ability to predict ACLF development, which warrants future investigations.

In conclusion, the results of our study indicate that organ dysfunction, including the liver, coagulation, kidney, and the presence of proven bacterial infection (cirrhosis-associated immune dysfunction), were associated with ACLF development within 28 days in AD patients. Patients with ≤1 dysfunctional organ at admission had a low risk of developing ACLF within 28 days. A simple algorithm (≤1 organ dysfunction) was able to safely rule out ACLF development within 4 weeks with a miss rate of <5%. Precise prediction of ACLF development in AD liver cirrhosis patients with ≥2 organ dysfunctions is warranted in future investigations.

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Conflict of interest

XZ, YG and YS have been editorial board members of *Journal of Clinical and Translational Hepatology* since 2021, 2019 and 2022 respectively. The other authors have no conflict of interest related to this publication.

Author contributions

Contributed to the study concept and design, acquisition of data, analysis and interpretation of data, and drafting of the

manuscript (XT), participated in the acquisition, analysis, and interpretation of the data (HL, GD, XZ, XW, YH, YG, ZM, ZQ, FL, XL, YS, BL, WG, JX, YX, YH, JC, NG, SL, LJ, JL, RZ, HR); contributed to the study concept and design, drafting of the manuscript, critical revision of the manuscript for important intellectual content, approval of the final version of the manuscript, statistical analysis, and study supervision and obtained funding (JC).

Ethical statement

Trial number of the validation cohort: NCT02457637 (www.ClinicalTrials.gov).

Data sharing statement

The datasets used in the study are available from the corresponding author on reasonable request.

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