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Cut-off level of serum caspase-cleaved CK 18 (M30) for diagnosis of acute on chronic liver failure in chronic hepatitis C cirrhosis

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Abstract

Background. *Acute on chronic liver failure (ACLF) is a severe form of liver cell failure with high mortality rates. Hepatocyte cell death in ACLF can occur through necrosis or apoptosis. The M30 fragment of caspase-cleaved cytokeratin 18 is a marker of caspase-mediated apoptosis.* ***Objective:*** *To assess serum caspase-cleaved CK 18 (M30) level as a diagnostic marker for acute-on-chronic liver failure (ACLF) in chronic hepatitis C cirrhosis patients. Furthermore, to determine the optimal cut-off level of serum caspase-cleaved CK 18 (M30) for diagnosing ACLF and its association with ACLF severity.* ***Methods:*** *The study involved 60 chronic HCV cirrhotic patients with ACLF (Group 1), 15 chronic HCV compensated cirrhotic patients (Group 2), and 15 healthy controls (Group 3). Laboratory tests and radiological assessments were conducted, including calculation of Child–Pugh score, MELD score, Consortium Liver Failure-Organ Failure (CLIF-OF) score, and CLIF-C-ACLF score using the EASL calculator. Serum level of caspase-cleaved CK 18 (M30) was measured using ELISA.* ***Results:*** *The serum level of caspase-cleaved CK 18 (M30) was significantly higher in group 1 compared to groups 2 and 3. Serum caspase-cleaved CK 18 (M30) showed a high predictive value for ACLF. At a cut-off level of 3.45 ng/ml, caspase-cleaved CK 18 (M30) had a sensitivity of 85% and specificity of 93.3% and AUC of 0.902 (95% CI = 0.833–0.970). There was a positive correlation between serum caspase-cleaved CK 18 (M30) levels and CLIF-C-ACLF score, indicating its potential as a predictor of ACLF severity.* ***Conclusion.*** *Serum caspase-cleaved CK 18 (M30), at cut off value of 3.45 ng/ml can accurately predict acute-on-chronic liver failure in patients with chronic HCV cirrhosis. Higher levels were associated with increased ACLF severity and mortality.*

Introduction

Acute on chronic liver failure (ACLF) is a newly identified disease entity characterized by acute deterioration of liver function in patients with chronic liver disease[1]. The development of liver failure and end organ dysfunction in ACLF is faster than chronic liver decompensation. ACLF patients may still have a chance of recovery of liver function[2]. Internationally, ACLF is defined as acute deterioration of preexisting chronic liver disease due to precipitating event with multisystem failure and increased 3-month mortality. The event may cause direct liver injury, for example, drug induced liver injury and viral hepatitis or may be extra hepatic such as surgery and infection. In some patients, it may be vague. The exact pathogenesis remains unclear. In ACLF, hepatocytes are exposed to high cytokine levels as Tumor necrosis factor-α (TNF-α), Interleukin-1, Lipopolysaccharides (LPS) and Interferon-ϒ. These cytokines stimulate both survival and apoptotic pathways simultaneously depending on whether the balance will tip to pro-apoptotic or anti-apoptotic pathway[3]. Apoptosis leads to the activation of caspase 3, which cleaves various substrates in the cell, including cytokeratin 18 (CK18), an important intermediate filament protein that plays a role in the morphological changes during hepatocyte apoptosis [4]. The full-length form of CK18 is released from necrotic cells, while a caspase-cleaved fragment is generated because of structural changes in apoptosis[5,6]. Soluble total CK18 and its fragments can be detected in human serum using immunoassays. The M65 assay measures both full-length CK18 and caspase-cleaved fragments generated through cell necrosis and apoptosis, although the M30 assay specifically detects a neoepitope formed in the caspase-3 cleaved 30kDa fragment during cell apoptosis [7,8]. The objective of this study is to assesses the utility of serum caspase-cleaved CK 18 (M30) levels in diagnosing and determining the severity of ACLF in patients with chronic HCV cirrhosis. Additionally, the study seeks to identify the most effective serum caspase-cleaved CK 18 (M30) cut-off level for diagnosing ACLF and its relationship with the severity of the condition.

Patients and Methods

This prospective study included 60 patients with chronic hepatitis C cirrhosis and acute-on-chronic liver failure (ACLF) (Group 1), 15 patients with chronic hepatitis C compensated cirrhosis (Group 2), and 15 healthy controls (Group 3) from September 2015 to September 2016. Patients were selected from the Hepatology and Gastroenterology unit and intensive care unit (ICU) at the Specialized Medical Hospital of the Faculty of Medicine at Mansoura University.

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Exclusion criteria

Involved, patients aged 70 years or older, those with heaptocellular carcinoma (HCC) under treatment, metastatic liver disease, cholangiocarcinoma, combined chronic hepatitis C and B, iatrogenic cirrhosis and fulminant liver failure (non-cirrhotic patients). All participants underwent a thorough history taking and clinical examination.

Laboratory and radiological tools

Included liver tests (ALT, AST, serum albumin, bilirubin, and INR), serum creatinine, complete blood count (CBC), serum electrolytes (sodium and potassium), C-reactive protein (CRP), ascitic fluid analysis for total and differential leukocyte count, urine analysis, and viral hepatitis markers using ELISA technique for hepatitis A, B, and C, as well as quantitative PCR for HCV. All patients underwent pelvic-abdominal ultrasonography, with contrast-enhanced computed tomography performed as needed. Diagnosis of liver cirrhosis was established based on clinical, laboratory, imaging, and previous endoscopic or biopsy findings.

Serum levels of cytokeratin 18 fragment M30 (CK 18-M30) antigen measurement

Serum levels of Cytokeratin 18 fragment (M30) antigen were measured using an ELISA kit provided by Sun Red PELOBIOTECH, Germany. This kit was used to detect CK 18-M30 in human serum or plasma. The kit utilizes a double antibody sandwich technique for ELISA. Peripheral venous blood samples were collected under aseptic conditions by venipuncture. Four milliliters of blood were placed in a plain tube and left to clot at room temperature for 10-20 minutes. The sample was then centrifuged at 2000-3000 rpm for 20 minutes, and the supernatant was stored at -20°C until testing. The assay could detect levels ranging from 0.3 to 30 ng/ml.

Scoring systems

Scoring systems were applied, included, Child-Turcotte-Pugh (CTP) score[9,10] Model of End-Stage Liver Disease (MELD) score[11], the Consortium Liver Failure-Organ Failure (CLIF-OF) score[12], and the CLIF-C-ACLF score[13]. Organ Failures (OF) were classified using the CLIF-SOFA score[14], a modified Sequential Organ Failure Assessment (SOFA) score. As a result, the necessity for renal replacement treatment or a blood creatinine level of ≥2.0 mg/dl were considered indicators of renal failure. A bilirubin level of ≥12.0 mg/dl was considered indicative of liver failure. Hepatic encephalopathy of grade III /IV was considered a sign of cerebral failure. A platelet count of $<20\times10^9$/l or an INR >2.5 were deliberated indicators of coagulation failure. The usage of vasopressors, like dopamine or terlipressin, was defined as a sign of circulatory failure. A PaO2/FiO2 ratio of less than 200 or a SpO2/FiO2 ratio less than 200 was considered respiratory failure. Acute-on-Chronic Liver Failure (ACLF) was defined according to EASL criteria[15]. ACLF was graded into three categories based on the number of organ failures: grade 1 comprised three subgroups of patients; single kidney failure; single failure of the liver, coagulation, circulation, or respiration with a serum creatinine level of 1.5 to 1.9 mg/dl, and cerebral failure (grade 3/4 hepatic encephalopathy) with a serum creatinine level between 1.5 and 1.9 mg/dl; grade 2 involved patients with two organ failures; and grade 3 involved patients with three or more organ failures.

Ethical approval

The Mansoura Medical Ethics Committee at the Faculty of Medicine approved the MD.15/05/109. Written consent was obtained from all patients or their families who participated in the study.

Statistical analysis

The collected data were analyzed using the Statistical Package for Social Science (SPSS) program for Windows (version 22). One-sample Kolmogorov-Smirnov test, Chi-square test, Student t-test, and Mann-Whitney test were employed as appropriate. Specificity and sensitivity at different cutoff points of CK 18-M30 levels for predicting ACLF were assessed using the receiver operating characteristic (ROC) curve. Logistic regression analysis was utilized to identify the most significant determinants of ACLF, while Cox regression analysis was employed to predict ACLF mortality. The significance threshold was set at 5%.

Results

Table 1 shows no differences as regardes, gender, age, diabetes, hypertension and neutrophil count among the three groups. Group 1 had significantly lower hemoglobin levels compared to groups 2 and 3. Leucocyte count was higher in group 1 versus groups 2 and 3. Lymphocyte count was significantly lower in group 1. Neutrophil-lymphocyte ratio (NLR) was higher in-group 1 compared to groups 2 and 3. Platelet count was lower in group 1 verus in groups 2 and 3. Group 1 had higher levels of serum AST, ALT, bilirubin, INR, creatinine, CRP, and K, and lower levels of serum albumin, PaO2, and serum Na compared to groups 2 and 3. CTP score was higher in group 1, with all cases in group 1 classified as CTP class C and all cases in group 2 as CTP class A. MELD score was also higher in group 1 compared to group 2. The serum level of M30 was higher in group 1 compared to groups 2 and 3. **Table 2** presents the precipitating factors of ACLF, with infection being the most common factor (61.7%). The most prevalent infection was SBP (30%), while hepatitis A and drug-induced liver injury were the least common precipitating factors. In group 1, 3.3% had single organ failure, 41.7% had two organ failures, and 55% had three or more organ failures. The most common organ failure in group 1 was renal failure (81.7%), followed by cerebral failure (78.3%), liver failure (38.3%), coagulation failure (23.3%), respiratory failure (11.7%), and circulatory failure (8.3%). ACLF grade 1 was present in 5%, grade 2 in 30%, and grade 3 in 65% of group 1 cases. The CLIF-OF score was 13.02 ± 1.97 (8-18) points, and the CLIF C ACLF score was 61.67 ± 9.31 (51-81) points in group 1. **Figure 1** illustrates that 18.5% of ACLF patients died on the index day, 48% on the third day, 73% on the seventh day, 85% on day 14, and 100% on day 28. The median survival time of ACLF cases was 8.48 ± 1.18 days, with a 95% confidence interval (CI) of 6.15-10.81. **Figure 2** demonstrates that the serum level of CK 18-M30 was significantly higher in group 1 compared to groups 2 and 3 (p-value < 0.05). **Table 3** shows no correlation between serum M30 level and demographic and laboratory variables, except for a significant positive correlation between serum CK 18-M30

level and CLIF C ACLF score. **Table 4** and **Figure 2** display the area under the ROC curve (AUC) for serum CK 18-M30 in predicting ACLF in Chronic HCV. At a cutoff point of 3.45 ng/ml, CK 18-M30 had optimal discriminative power for predicting Chronic HCV patients, with a sensitivity of 85%, specificity of 93.3%, positive predictive value (PPV) of 98.1%, negative predictive value (NPV) of 60.8%, and an accuracy of 86.7%.

Table 1. Demographic, clinical and laboratory data of the studied patients.

Variables	Group 1 (n=60) No (%)	Group 2 (n=15) No (%)	Group 3 (n=15) No (%)	*P1*	*P2*	*P3*
Gender						
▪ ***Male***	35 (58.3%)	6 (40%)	5 (33.3%)	0.2	0.085	0.71
▪ ***Female***	25 (41.7%)	9 (60%)	10 (66.7%)			
Age/years (Mean ± SD)	56.85±7.50	54.27±7.46	53.0±9.58	0.24	0.097	0.68
Diabetes mellitus	26 (43.3%)	7 (46.7%)	3 (20%)	0.816	0.096	0.121
Hypertension	8 (13.3%)	4 (26.7%)	2 (13.3%)	0.208	1.0	0.651
Hemoglobin(g/dl)	9.96±2.31	12.41±1.95	12.28±1.65	<.001	0.005	0.841
Leucocytes $\times 10^9$/L	15.79±9.45	6.91±2.69	8.13±2.76	0.001	0.003	0.23
Neutrophils 10^9/L	64.71±31.08	77.73±6.07	80.60±5.46	0.11	0.054	0.185
Lymphocytes 10^9/L	5.00 (0.6-91)	10 (6-12)	8 (5-11)	0.007	0.056	0.376
NLR	15.41±1.74	8.68±2.01	10.22±3.04	0.005	0.05	0.114
platelets $\times 10^9$/L	74.50 (16-556)	174(62-315)	200 (156-377)	<.001	<.001	0.146
AST(U/L)	128.5 (21-4500)	55 (11-89)	24 (11-78)	<.001	<.001	0.12
ALT(U/L)	64 (15-1240)	38 (13-79)	29 (13-63)	0.012	0.001	0.32
Bilirubin (mg/dl)	10.85 (0.7-37.50)	1 (0.3-1.7)	0.8 (0.4-1.1)	<.001	<.001	0.155
INR	2.24±0.78	1.03±0.13	0.98±0.07	<.001	<.001	0.21
Creatinine (mg/dl)	3.36±1.76	1.02±0.19	0.93±0.20	<.001	<.001	0.21
CRP (mg/l)				<.001	<.001	0.014
▪ ***Positive***	58 (96.7%)	5 (33.3%)	0 (0.0%)			
▪ ***Negative***	2 (3.3%)	10 (66.7%)	15(100%)			
Albumin(g/dl)	2.23±0.47	3.76±0.75	3.94±0.28	<.001	<.001	0.38
PaO2 (mmHg)	53.31±16.00	83.87±6.33	86.60±5.91	<.001	<.001	0.23
Na (mEq/l)	123.70±10.99	132.73±3.65	134.73±5.07	0.003	<.001	0.22
K (mEq/l)	4.56±1.05	3.90±0.48	3.79±0.41	0.02	0.007	0.51
CTP score (M ± SD)	13.31±1.73	5.47±0.51	-	0.001		
CTP class (A/B/C)	0/0/60 (0/0/100%)	15/0/60 (100/0/0%)	-	-	-	-
MELD score (M ± SD)	32.32±6.76 (19- 46)	7.67±2.06	-	<.001	-	-
M30 (ng/ml)	13.1 (2-31.8)	5.7 (3.0-13.6)	3.0 (1.6-4.1)	0.017	<.001	<.001

NLR: *Neutrophil-lymphocyte ratio);* ***P1:*** *Group 1 VS Group 2;* ***P2:*** *Group 1 VS Group 3;* ***P 3:*** *Group 2 Group 3.*

Table 2. Precipitating factors of ACLF.

Precipitating factor	No & %
Infection	37 (61.7%)
SBP	18 (30.0%)
Urinary tract infection	9 (15%)
Pneumonia	9 (15%)
Abscess (gluteal)	1(1.7%)
Upper GIT bleeding	21(35%)
Hepatitis A virus infection	1(1.7%)
Drugs (direct acting anti-viral)	1(1.7%)

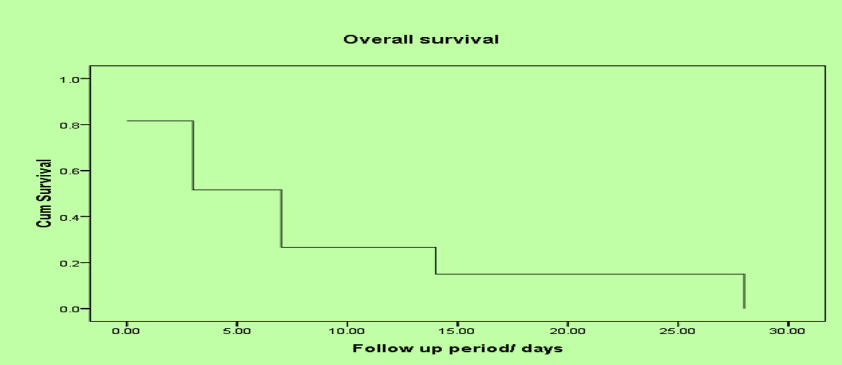

Figure 1. Cumulative mortality of ACLF patients

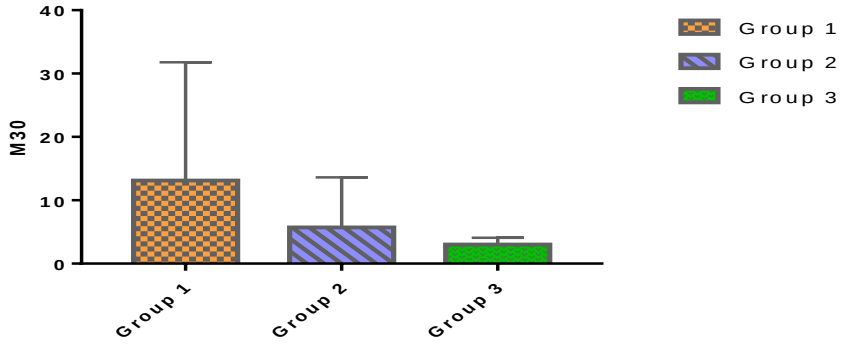

Figure 2. Serum level of CK 18-M30 in the studied groups.

Table 3. Correlation between serum M30 level and demographic and laboratory variables.

	Serum M30 level	
	R	***P***
Age/years	0.113	0.388
Hemoglobin (gm/dl)	0.128	0.332
Leucocytes×109/L	0.091	0.489
PLC×10^9/L	0.024	0.853
Neutrophils×10^9/L	-0.065	0.621
Lymphocytes×109/L	0.070	0.593
NLR	-0.173	0.185
SGPT(IU/L)	-0.101	0.441
SGOT(IU/L)	0.068	0.607
Bilirubin (mg/dl)	0.039	0.765
Albumin (gm/dl)	0.217	0.095
INR	0.188	0.151
Creatinine (mg/dl)	-0.173	0.185
CRP	-0.040	0.760
Serum Na (mmol/l)	0.052	0.693
Serum K(mmol/l)	-0.136	0.302
PaO2	-0.034	0.798
Grade of ACLF	0.194	0.137
CLIF OF score	0.171	0.193
CLIF C ACLF score	0.331	0.01
CTP score	0.120	0.362
MELD score	0.013	0.921

Table (4). Level and area under ROC of serum CK 18-M30 for prediction of ACLF patients.

Item	AUC	95% Confidence Interval		Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
		Lower Bound	*Upper Bound*						
M30	0.902	0.833	0.970	>3.45	85%	93.3%	98.1%	60.8%	86.7%

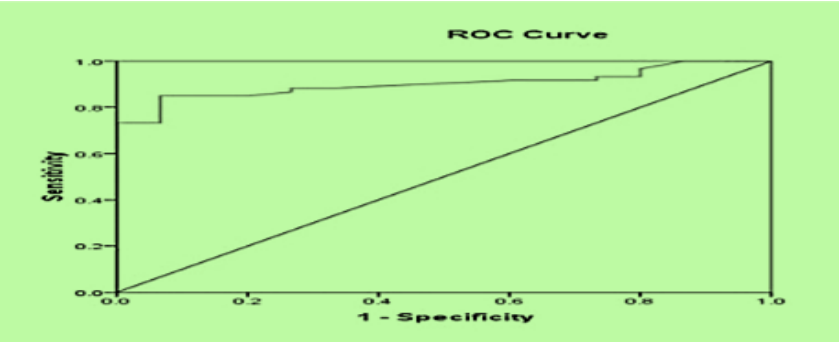

Figure 3. Receiver operator characteristics curves for serum level of CK 18-M 30 in prediction of ACLF.

Discussion

Our study found a significantly higher serum level of CK 18-M30 in ACLF patients compared to non-ACLF and healthy groups. This result aligns with previous research showing elevated levels of cytokeratin 18 (M30) in ACLF patients on immunohistochemistry staining of liver biopsy samples, indicating increased hepatic expression and suggesting that apoptosis is the main mode of hepatocyte death in ACLF patients[16,17]. In addition Zheng et al.'s discovered increased levels of CK 18-M30 and CK 18- M65 fragments in ACLF patients and suggesting that the M30/M65 ratio may serves as a valuable indicator for evaluating the heaptocyte apoptosis-to-necrosis balance in liver conditions[18]. In this study, we discovered a notable positive correlation between serum CK 18-M30 levels and CLIF C ACLF score, aligning with prior research that identified serum CK 18-M30 levels as an independent predictor of ACLF and associated mortality. This reinforces the notion that CK 18-M30 levels can serve as an indicator of the severity of liver disease in ACLF[16]. This study declared that CK 18-M30 at a cutoff point of 3.45 ng/ml had optimal discriminative power for predicting ACLF patients, with a sensitivity of 85%, specificity of 93.3%, PPV of 98.1%, NPV of 60.8%, and an accuracy of 86.7%. Similarly, Zheng et al. reported that CK 18-M30 at a cutoff of 333.35 U/L had an AUC of 0.80 (95% CI: 0.68-0.92), with a sensitivity of 96.7% and specificity of 71.0% for identifying ACLF patients in chronic hepatitis B infection [18]. Additionally, Church et al. found that elevated serum CK 18-M30 levels were significant predictors of death or liver transplantation, with an ROC AUC of 0.832 (95% CI 0.737-0.927) for full-length CK18 and an ROC AUC of 0.778 (95% CI 0.676-0.881) for caspase-cleaved (cc)[19]. Our study found no correlation between serum CK 18-M30 levels and most study variables, except for a significant positive correlation between serum CK 18-M30 levels and the CLIF C ACLF score. However, a previous study indicated a strong correlation between M30 levels and ALT and AST levels in both ACLF and CHB patients[18]. Additionally, Adebayo et al. reported a significant correlation between M30 levels and the number of organ failures in patients with acute decompensation of cirrhosis. The area under the curve for predicting mortality was 0.88. Using a CK 18-M30 cut-off value of 1312, the sensitivity was 83% and the specificity was 72% for predicting mortality. Furthermore, in ACLF patients with ongoing infections, a correlation was observed between high levels of circulating CK 18-M30 and the presence of infections[16]. In this study, it was found that infection was the main trigger for ACLF, responsible for 61.7% of cases, with spontaneous bacterial peritonitis being the most common type. This aligns with earlier research indicating that infection is a significant factor in the development of ACLF[20]. In addition, Hernaez et al. demonstrated that systemic inflammation and organ failure in patients with ACLF were often caused by bacterial infections, with SBP being the most common infection leading to sepsis-induced ACLF[21]. The study revealed that upper gastrointestinal bleeding was the second most common precipitating factor of ACLF, accounting for 35% of cases. This is consistent with the CANONIC study, which found that acute variceal bleeding was a precipitating event in 13.8% of ACLF patients. Acute variceal bleeding can lead to hepatic ischemia, triggering ACLF[22,23]. Additionally, acute hepatitis A infection and the use of direct-acting antivirals for chronic hepatitis C were identified as precipitating factors in 1.7% of cases each[24]. The study found that 3.3% of ACLF patients had single organ failure, 41.7% had two organ failures, and 55% had three or more organ failures. ACLF grade 1 was present in 5% of patients, grade 2 in 30%, and grade 3 in 65%. These results differ from a study by Rita Barosa et al, which showed that most ACLF patients had grade 1 and grade 2 ACLF, with a minority having grade 3 ACLF. Renal failure was the most common organ failure in ACLF patients, with causes including hepatorenal syndrome, parenchymal disease, hypovolemia-induced, or drug-induced renal failure. Cerebral failure was the second most common organ failure in this study (78.3%), possibly due to brain swelling, a significant feature of ACLF. These findings contrast with the CANONIC study, which identified renal, liver, cerebral, and coagulation failures as the most common organ failures in ACLF patients[22]. The study found that all patients in the ACLF group were classified as CTP class C, consistent with previous research[25]. Additionally, the ACLF group had significantly higher MELD scores, which have been shown to predict prognosis in ACLF patients[26]. The mortality rates in the ACLF group were 18.5% on day zero, 48% on day 3, 73% on day 7, 85% on day 14, and 100% on day 28. This aligns with previous findings that ACLF is a dynamic condition with a high mortality rate, especially in the short term[21,27]. Our study showed lower hemoglobin levels, higher total leukocyte counts, elevated NLR, lower PLC, elevated AST, bilirubin, INR, CRP levels, and lower

albumin levels in ACLF group compared to other groups. These results support earlier studies linking these parameters to ACLF and its prognosis. Hyperkalemia and hyponatremia were also observed in ACLF patients, likely due to various factors such as poor intake, gastrointestinal issues, and medication use [28,29-32]. One limitation of this study was the absence of liver biopsy to correlate serum CK18-M30 levels with liver pathology. Future studies with larger sample sizes encompassing various causes of liver cirrhosis are needed to validate the findings of this study.

Conclusion

Serum cytokeratin 18 (M30) can effectively predict acute-on-chronic liver failure in patients with chronic HCV cirrhosis. Elevated CK18-M30 levels were linked to greater ACLF severity based on the CLIF-C-ACLF score and served as a robust predictor of mortality in ACLF.

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