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Remnant cholesterol is associated with poor prognosis in patients with hepatitis B-related acute-on-chronic liver failure: a Chinese population-based study

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Abstract

Background Hepatitis B-related acute-on-chronic liver failure (HBV-ACLF) patients possess adverse lipid homeostatic alterations, subsequently affecting their treatment regimens and prognoses. However, the precise association between one lipid homeostasis indicator, remnant cholesterol (RC), and HBV-ACLF prognoses have not been fully elucidated. In this retrospective study, the relationship between RC with 28- and 90-day HBV-ACLF prognoses was delineated.

Methods 595 HBV-ACLF patients were recruited, and data collected for laboratory parameters at admission, as well as whether poor 28- and 90-day prognoses occurred during the follow-up period, in the form of mortality, or liver transplantation. Patients were divided into 3 groups, based on RC tertiles (Q1-3), and 4 multivariate Cox regression analyses were conducted to identify the associations between RC levels and ACLF prognoses; these analyses excluded different confounding factors, based on the Strengthening the Reporting of Observational Studies in Epidemiology statement. Stratified analysis was conducted to investigate the association between RC and ACLF risk among different subgroups, based on age, sex as well as complications and artificial liver treatment. RC accuracy versus that of other lipid indicators to predict 28- and 90-day ACLF survival was evaluated by restricted cubic spline and receiver operating characteristic (ROC) curve analyses, while Kaplan-Meier curves measured cumulative 28- and 90-day mortality risks.

Results For all 4 regression models, higher RC were associated with worse liver function, coagulation, and HBV-ACLF prognoses. Restricted cubic spline analysis identified a non-linear relationship between RC and HBV-ACLF prognoses, in which the Q3 RC tertile had the lowest 28-day and 90-day HBV-ACLF survival rates; this was further confirmed by Kaplan-Meier analysis. Additionally, subgroup analysis found that higher RC correlated to worse ACLF prognoses among hypoproteinemia patients. Moreover, RC, compared to total cholesterol, triglycerides, high- and low-density lipoprotein cholesterol, as well as non-high density lipoprotein, was the most accurate in predicting poor 28- and 90-day ACLF prognoses.

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Conclusions Elevated RC was significantly associated with poorer 28- and 90-day HBV-ACLF prognoses, even after accounting for all other traditional risk factors. Therefore, monitoring RC, along with interventions to reduce their levels, could aid in improving ACLF patient outcomes.

Keywords Acute-on-chronic liver failure, Prognosis, Remnant cholesterol, 28-day survival rate, 90-day survival rate

Background

Acute-on-chronic liver failure (ACLF) is characterized by acute deterioration of liver function, manifesting as jaundice, coagulopathy, ascites, and high 28-day mortality rates [1]. Although the artificial liver support system can improve short-term prognoses for ACLF patients, survival rates are still low [2–4]. Therefore, early assessment of patient prognoses can help guide clinicians in formulating effective treatment strategies. In recent years, measuring lipid indicators have shown great potential as an ACLF prognostic assessment tool, of which one of the most significant is remnant cholesterol (RC), defined as total cholesterol (TC) minus high-density (HDL-c) minus low-density lipoprotein cholesterol (LDL-c). Thus, RC is mainly composed of very low- and intermediate-density lipoproteins, in the fasting state, as well as those 2 lipoprotein types with chylomicron remnants in the non-fasting state [5–6]. Owing to RC comprising of cholesterol-rich lipoproteins, it promotes fat accumulation in the liver, as well as being able to induce local inflammation, via penetrating the liver endothelium and activating Kupffer cells [7]. With respect to liver diseases, recent studies have shown that higher RC levels were associated with increased non-alcoholic fatty liver disease (NAFLD), as well as being predictive for complicating cardiovascular and cerebrovascular events among NAFLD individuals [8–9]. Therefore, high RC has been observed to be correlated with liver dysfunction and its associated complications. However, the relationship between RC and ACLF prognoses are still largely unknown. Consequently, this retrospective study aimed to shed light on whether RC was linked to hepatitis B-related (HBV)-ACLF, by identifying the presence of associations between RC levels with 28- and 90-day ACLF prognoses. It was found that higher RC levels were predictive for worse ACLF prognoses, particularly for those who also had hypoproteinemia. This suggests that RC could serve as a potential diagnostic marker for identifying HBV-ACLF patients with worse survival outcomes, thereby facilitating personalized treatment approaches to improve their survival prospects.

Methods

Patient recruitment, inclusion, and exclusion criteria

A total of 595 HBV-ACLF patients, admitted to the Department of Infectious Diseases at the First Affiliated Hospital of Nanchang University, between January 2013-December 2023, were enrolled for this retrospective study. Inclusion criteria was based on ACLF diagnosis in accordance with the following Asia-Pacific Association for the Study of the Liver criteria: (1) Severe jaundice, defined by serum bilirubin (Bil) ≥ 5 mg/dl, as well as (2) coagulopathy, defined as international normalized ratio ≥ 1.5 or prothrombin activity ≤ 40%, along with having (3) Previously un- or diagnosed HBV-associated chronic liver disease, as well as (4) Presence of hepatic encephalopathy and/or ascites ≤ 4 weeks pre-hospitalization. Exclusion criteria were: (1) Other chronic liver and/or systemic diseases, such as recent alcohol-related liver damage, autoimmune or metabolic liver disease, that are not linked to HBV infection (ex. hepatitis C or D-linked liver disease), as well as cancer, HIV, previous renal insufficiency or heart failure, (2) Pregnant or lactating female, (3) Incomplete clinical data available, or (4) Patient lost to follow-up or automatic discharge.

Data collection, biomarker measurement, and classifying HBV-ACLF prognoses

Patient data were obtained from electronic medical records. Additionally, fasting venous blood was collected from all patients during hospitalization; serum ALT, aspartate aminotransferase (AST), as well as total (TBil) and direct Bil (DBIL), plus albumin (ALB), γ-glutamyl transferase (GGT), blood urea nitrogen (BUN), fasting plasma glucose (FPG), TC, triglycerides (TG), HDL-c, LDL-c, RC, Na^+, prothrombin time (PT), white blood cells (WBC), and platelets (PLT), were measured. Patient follow-up was carried out by telephone after 28 and 90-days post-hospitalization, in line with previous studies, such as by Song et al. [10] and Zhou et al. [11], as they are standardized time points for examining the effects of factors affecting ACLF patient prognoses. Poor ACLF prognoses were defined as the occurrence of either mortality or liver transplantation during those periods.

Statistical analyses

All statistical analyses were performed using R software (version 3.4.3). RC were divided into tertiles, and parameters for each tertile were expressed as percentages for categorical variables. Continuous variables were expressed as mean ± standard deviation, if they had a normal distribution, or median (interquartile ranges), if their distribution was non-normal. Comparisons between 3 or more groups were conducted using either the χ^2 test for categorical variables, or one-way analysis of variance (ANOVA), followed by the Kruskal-Wallis test

for continuous variables. $P<0.05$ was considered statistically significant.

To further identify the presence of associations between RC levels and HBV-ACLF prognoses, 4 multivariate Cox regression analyses were conducted, indicated as Models 1–4. Model 1 represents the initial multivariate Cox regression analysis, 2 excluded sex and age as confounding factors, 3 excluded sex, age, as well as the laboratory parameters FPG, PLT, ALT, AST, ALB, GGT, Na^+ and PT, plus infection, liver cirrhosis, hypoproteinemia, and artificial liver treatment as confounding factors. Model 4 was the fully adjusted model, excluding all non-collinear co-variates, comprising of parameters excluded in Model 3, along with WBC, TBil, BUN, hepatic encephalopathy, hepatorenal syndrome, and diabetes as confounding factors. Hazard ratios (HRs) and 95% confidence intervals (CI) were measured for all models. The choice of confounding factors to exclude for these analyses were identified based on the guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology statement [12]. The presence of a potential non-linear relationship between RC and HBV-ACLF prognoses was then evaluated by restricted cubic spline (RCS) analysis, the cumulative risk of death during the 28- and 90-day evaluation periods was analyzed by Kaplan-Meier curves, followed by verification with the log-rank test.

Furthermore, stratified analysis was performed to examine the association between RC and HBV-ACLF prognoses among different subgroups, based on age, sex, complications, and artificial liver treatment, and the potential differences in those associations among those groups were further evaluated by the likelihood ratio test. To measure the accuracy of RC and other lipid indicators to predict 28- and 90-day survival for HBV-ACLF, receiver operating characteristic (ROC) curve was used.

Results

Clinical characteristics of the subjects

Among the 595 HBV-ACLF patients, 517 (86.89%) were male, and average age was 43 years. All of them had worse liver function and coagulation indicators, compared to healthy individuals, consistent with having clinical liver failure. With respect to blood lipid indicators, they were, respectively, 2.55 (2.17, 2.96), 1.10 (0.79, 1.53), 1.16 (0.42, 1.29) and 1.15 (0.69, 1.62) mmol/L for baseline TC, TG, HDL-c, and LDL-c for the whole study population. The study population was then divided into 3 groups, based on RC tertiles: 0-0.24 (Q1), 0.24–0.31 (Q2), and 0.31–0.56 (Q3), and baseline clinical characteristics for each group were shown in Table 1. Among the 3 groups, Q3 had the highest TC, TG, and LDL-c, as well as the lowest HDL-c levels (Table 1). Q3 also had the worst liver function and coagulation indicators, in the form of the highest levels of ALT, AST, GGT, ALB and PT, compared to Q1 and 2 (Table 1). This observation corresponded to Q3 having the greatest proportion of patients with poor ACLF prognosis, at 60.41%, compared to Q1 at 8.59% and Q2 at 40.50% (Table 1). Additionally, Q3 had the highest percentages of individuals with adverse complications, such as infection, liver cirrhosis, and hypoproteinemia, compared to Q1 and Q2, as well as being the most likely to undergo artificial liver treatment (Table 1). All these findings thus indicate that lipid metabolism may be associated with ACLF prognoses and complications; in particular, higher RC levels are associated with worse liver function and coagulation, along with poorer HBV-ACLF prognoses and greater likelihood for complications.

Associations between RC and the risk of adverse prognosis in patients with HBV-ACLF

The relationship between RC levels and risk for poor HBV-ACLF prognoses was then examined by 4 multivariate Cox regression models, as shown in Table 2. For Model 1, which did not have any patient parameters excluded as confounding factors, significant differences were observed for Q2 and Q3 versus Q1, which was used as the reference, in which Q3 had the highest HR (95% CI), at 9.85 (5.92 ~ 16.39), compared to 6.06 (3.59 ~ 10.23) for Q2. Similar results were also observed for Models 2, which excluded sex and age as confounding factors, 3, which excluded sex, age, and laboratory indicators FPG, PLT, ALT, AST, ALB, GGT, Na^+, PT, plus infection, liver cirrhosis, hypoproteinemia, and artificial liver treatment, as well as for 4, which excluded the same factors as 3, along with WBC, TBil, BUN, hepatic encephalopathy, hepatorenal syndrome, and diabetes; all of those models had Q3 possess the highest HR (Table 2). Some factors, like TBil and BUN were not significantly different among Q1-3 (Table 1), but they were included in Models 3 or 4 owing to them being considered as potential independent predictors for ACLF prognoses in previous studies [13–15]. Ultimately, though, these results thus illustrate that HBV-ACLF patients with higher RC levels were more likely to have poorer prognoses for the disease.

The association between higher RC levels and poorer HBV-ACLF prognoses was further verified by RCS analysis, which revealed a non-linear relationship (Fig. 1). Model fit was then assessed by AIC, in which the best number of nodes in the RCS curve was 4, as the 1st node yields a value of 0.130 in the 5th quantile, the 2nd node 0.243 in the 35th quantile, the 3rd node 0.308 in the 65th quantile, and the 4th node 0.430 in the 95th quantile.

Additionally, Kaplan-Meier curve analyses further demonstrated that the highest RC tertile (Q3) was associated with the lowest 28- and 90-day survival rates for ACLF, compared to Q1 and Q2 (Fig. 2A-B; $P<0.001$).

Table 1 Baseline characteristics for patients with hepatitis B-related acute-on-chronic liver failure (HBV-ACLF) among the 3 remnant cholesterol (RC) tertiles variables

	Total (*n* = 595)	Q1 (0-0.24)	Q2 (0.24–0.31)	Q3 (0.31–0.56)	*P*
Sex					0.003
Male	517 (86.89)	168 (84.85)	165 (82.50)	184 (93.40)	
Female	78 (13.11)	30 (15.15)	35 (17.50)	13 (6.60)	
Age (years)	43.00 (34.00, 51.00)	42.00 (34.00,49.00)	44.00 (35.00,51.00)	43.00 (34.00,51.00)	0.433
FPG (mmol/L)	4.08 (3.33, 5.83)	4.10 (3.33,6.04)	3.98 (3.25,5.69)	4.22 (3.41,5.82)	0.292
TC (mmol/L)	2.55 (2.17, 2.96)	2.10 (1.73,2.42)	2.58 (2.28,2.86)	3.01 (2.67,3.35)	< 0.001
TG (mmol/L)	1.10 (0.79, 1.53)	0.86 (0.64,1.13)	1.07 (0.79,1.43)	1.44 (1.11,1.78)	< 0.001
HDL-c (mmol/L)	1.16 (0.42, 1.29)	1.22 (1.13,1.33)	1.16 (0.39,1.28)	1.11 (0.24,1.23)	< 0.001
LDL-c (mmol/L)	1.15 (0.69, 1.62)	1.01 (0.60,1.32)	1.19 (0.69,1.61)	1.39 (0.80,1.88)	< 0.001
RC (mmol/L)	0.27 (0.22, 0.34)	0.19 (0.16,0.22)	0.27 (0.26,0.29)	0.36 (0.34,0.40)	< 0.001
WBC (10^9/L)	6.07 (4.77, 7.80)	5.74 (4.57,7.82)	6.46 (4.72,7.92)	6.03 (5.07,7.47)	0.385
PLT (10^9/L)	118.00 (82.00, 152.50)	106.50 (78.25,137.00)	126.50 (93.75,160.25)	119.00 (82.00,152.00)	0.002
ALT (U/L)	715.00 (261.50, 1338.00)	535.00 (188.75,1081.75)	732.55 (304.15,1340.53)	853.00 (409.00,1478.10)	< 0.001
AST (U/L)	444.00 (190.50, 947.80)	339.50 (145.00,736.85)	476.50 (228.60,1022.62)	486.00 (244.70,1027.00)	< 0.001
TBil (μmol/L)	257.30 (188.00, 356.35)	263.45 (188.93,334.70)	264.25 (190.05,375.83)	256.70 (179.70,369.50)	0.724
GGT (U/L)	97.00 (62.00, 142.00)	81.50 (52.00,120.50)	97.00 (63.50,142.50)	111.00 (73.00,166.00)	< 0.001
ALB (g/L)	32.80 (29.60, 35.60)	31.80 (29.40,34.85)	32.90 (29.55,35.73)	33.30 (31.00,36.10)	0.009
BUN (mmol/L)	3.30 (2.60, 4.50)	3.20 (2.50,4.47)	3.40 (2.70,4.30)	3.40 (2.80,4.60)	0.271
Na^+ (mmol/L)	138.00 (136.00, 140.00)	138.40 (137.00,140.52)	138.00 (135.67,140.10)	137.50 (134.10,139.60)	< 0.001
PT (seconds)	23.00 (19.60, 27.05)	23.75 (20.75,27.30)	23.10 (19.58,27.73)	21.30 (19.30,25.60)	0.001
Prognosis					
Recovery	378 (63.53)	181 (91.41)	119 (59.50)	78 (39.59)	< 0.001
Poor	217 (36.47)	17 (8.59)	81 (40.50)	119 (60.41)	
Hepatic encephalopathy					0.095
Yes	530 (89.08)	184 (92.93)	173 (86.50)	173 (87.82)	
No	65 (10.92)	14 (7.07)	27 (13.50)	24 (12.18)	
Infection					0.004
Yes	380 (63.87)	112 (56.57)	125 (62.50)	143 (72.59)	
No	215 (36.13)	86 (43.43)	75 (37.50)	54 (27.41)	
Liver cirrhosis					< 0.001
Yes	428 (71.93)	122 (61.62)	151 (75.50)	155 (78.68)	
No	167 (28.07)	76 (38.38)	49 (24.50)	42 (21.32)	
Hypoproteinemia					< 0.001
Yes	309 (51.93)	58 (29.29)	113 (56.50)	138 (70.05)	
No	286 (48.07)	140 (70.71)	87 (43.50)	59 (29.95)	
Artificial liver therapy					< 0.001
Yes	335 (56.30)	93 (46.97)	117 (58.50)	125 (63.45)	
No	260 (43.70)	105 (53.03)	83 (41.50)	72 (36.55)	
Diabetes					0.588
Yes	555 (93.28)	187 (94.44)	187 (93.50)	181 (91.88)	
No	40 (6.72)	11 (5.56)	13 (6.50)	16 (8.12)	
Hepatorenal syndrome					0.205
Yes	561 (94.29)	189 (95.45)	191 (95.50)	181 (91.88)	
No	34 (5.71)	9 (4.55)	9 (4.50)	16 (8.12)	

FPG Fasting plasma glucose *TC* Total cholesterol *TG* Triglycerides *HDL-c* High-density lipoprotein cholesterol *LDL-c* Low-density lipoprotein cholesterol *RC* Remnant cholesterol *WBC* White blood cell *PLT* Platelet *ALT* Alanine aminotransferase *AST* Aspartate aminotransferase *TBil* Total bilirubin *GGT* γ-glutamyl transferase *ALB* Albumin *BUN* Blood urea nitrogen *PT* Prothrombin time

Subgroup analysis of the relationship between RC and HBV-ACLF risk

We then further examined whether the association between higher RC levels and increased HBV-ACLF risk was present among different age, sex, as well as complications and artificial liver treatment subgroups (Table 3). The age subgroups were < 50 and ≥ 50 years, in which no significant differences were present, both before and after adjustments, the latter by applying the same exclusion of confounding variables as Model 3 (age,

Table 2 Multivariate Cox regression analyses of the association between RC tertiles and poorer HBV-ACLF prognoses, for different models

RC tertiles	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Q1	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Q2	6.06 (3.59 ~ 10.23)	< 0.001	6.06 (3.59 ~ 10.24)	< 0.001	6.25 (3.59 ~ 10.89)	< 0.001	6.30 (3.58 ~ 11.07)	< 0.001
Q3	9.85 (5.92 ~ 16.39)	< 0.001	10.24 (6.13 ~ 17.11)	< 0.001	12.11 (6.87 ~ 21.36)	< 0.001	11.02 (6.20 ~ 19.60)	< 0.001

HR Hazard Ratio *CI* Confidence Interval

Model 1 Unadjusted *Model 2* Adjusted for sex, age *Model 3* Adjusted for sex, age, FPG, PLT, ALT, AST, ALB, GGT, Na^+, PT, infection, liver cirrhosis, hypoproteinemia, artificial liver treatment *Model 4* Adjusted for sex, age, FPG, WBC, PLT, ALT, AST, TBil, GGT, ALB, BUN, Na^+, PT, hepatic encephalopathy, hepatorenal syndrome, infection, liver cirrhosis, hypoproteinemia, artificial liver treatment, diabetes

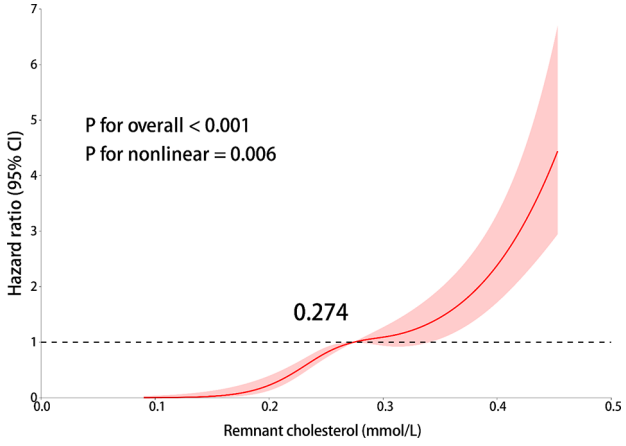

Fig. 1 Restricted cubic spline analysis plot showing a non-linear relationship between remnant cholesterol (RC) levels and risk of poor prognosis for hepatitis B-related acute-on-chronic liver failure (HBV-ACLF), after adjusting for sex, age, FPG, PLT, ALT, AST, ALB, GGT, Na^+, PT, infection, liver cirrhosis, hypoproteinemia, and artificial liver treatment (Model 3). Hazard ratio in the Y-axis represents the probability for poor HBV-ACLF prognoses, solid red line represents smooth curve fitting, and the light red area around the solid red line represents the 95% confidence interval

sex, FPG, PLT, ALT, AST, ALB, GGT, Na^+, PT, infection, liver cirrhosis, hypoproteinemia, artificial liver treatment). Similarly, no significant differences were found between male and female patient subgroups, both before and after adjustments (Table 3). This was further supported by the observation that out of the complications of infection, liver cirrhosis, and hypoproteinemia, as well as artificial liver treatment, only hypoproteinemia had a significantly higher HR post-adjustment, compared to those without hypoproteinemia, indicating its potentially significant influence on higher RC being associated with poorer HBV-ACLF prognoses.

The value of RC in predicting the risk of poor prognosis at 28 and 90 days for HBV-ACLF

To evaluate the predictive capabilities of lipid parameters, such as RC, for poorer HBV-ACLF prognoses, ROC curve analyses were carried out, as shown in Fig. 3A-B. It was found that RC had the highest area under the curve (AUC), at 0.777 (0.737–0.817), compared to other lipid parameters, such as TC, TG, HDL-c, LDL-c, and Non-HDL-c, for more adverse 28-day prognoses (Fig. 3A). The same result was also present for more adverse 90-day prognoses, in which RC also had the highest AUC, at 0.782 (0.744–0.819; Fig. 3B). Therefore, RC was the most accurate lipid parameter for predicting worse 28- and 90-day ACLF prognoses. Overall, RC could serve as a potential diagnostic parameter for identifying ACLF patients with worse medium- and long-term prognoses, in the form of lower survival rates, subsequently enabling personalized interventions for those patients.

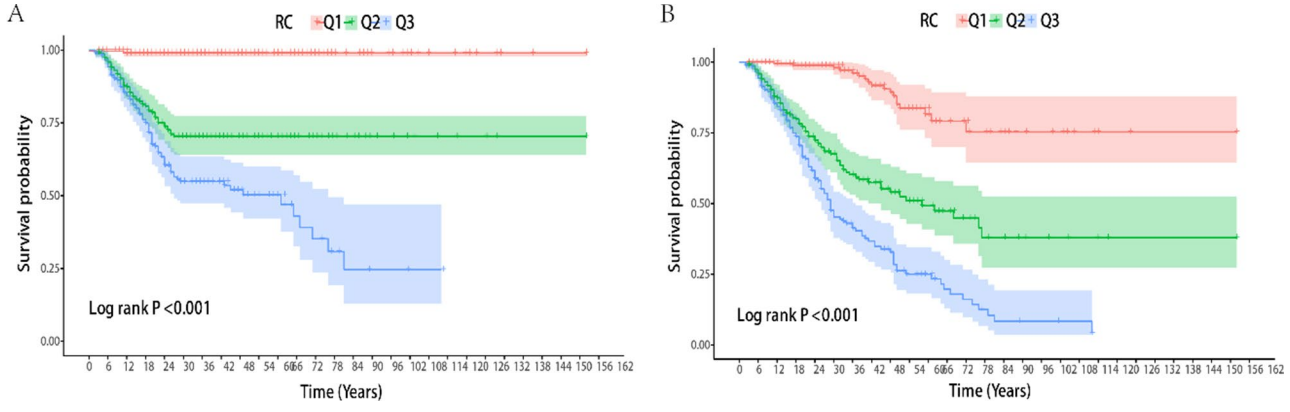

Fig. 2 Kaplan-Meier curves measuring the cumulative risk of death from HBV-ACLF after **A** 28- and **B** 90-days, for the 3 RC tertiles

Table 3 Stratified analysis of the association between RC and poorer HBV-ACLF prognoses, among different age, sex, infection, liver cirrhosis, hypoproteinemia, and artificial liver treatment subgroups

Variables	*n* (%)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	*P*	*P* (interaction)
Sex					0.649
Male	517 (86.89)	2.66 (1.94 ~ 3.65)	2.63 (1.83 ~ 3.76)	< 0.001	
Female	78 (13.11)	3.52 (1.55 ~ 7.97)	42.53 (6.43 ~ 281.40)	< 0.001	
Infection					0.157
No	380 (63.87)	2.44 (1.67 ~ 3.56)	2.12 (1.36 ~ 3.30)	< 0.001	
Yes	215 (36.13)	3.24 (2.03 ~ 5.18)	3.35 (1.99 ~ 5.63)	< 0.001	
Liver cirrhosis				< 0.001	0.871
No	428 (71.93)	2.64 (1.84 ~ 3.77)	2.66 (1.77 ~ 3.98)	< 0.001	
Yes	167 (28.07)	3.03 (1.79 ~ 5.12)	3.05 (1.64 ~ 5.67)	< 0.001	
Hypoproteinemia					0.045
No	309 (51.93)	1.96 (1.30 ~ 2.97)	1.91 (1.21 ~ 3.02)	0.005	
Yes	286 (48.07)	3.28 (2.12 ~ 5.08)	4.04 (2.43 ~ 6.70)	< 0.001	
Artificial liver treatment					0.761
No	335 (56.30)	2.49 (1.63 ~ 3.80)	2.30 (1.42 ~ 3.71)	< 0.001	
Yes	260 (43.70)	2.72 (1.79 ~ 4.13)	3.47 (2.11 ~ 5.70)	< 0.001	
Age (years)					0.915
< 50	429 (72.10)	2.61 (1.81 ~ 3.76)	2.64 (1.72 ~ 4.04)	< 0.001	
≥ 50	166 (27.90)	2.71 (1.66 ~ 4.43)	3.43 (1.91 ~ 6.15)	< 0.001	

HR Hazard Ratio *CI* Confidence Interval

Adjusted for sex, age, FPG, PLT, ALT, AST, ALB, GGT, Na^+, PT, infection, liver cirrhosis, hypoproteinemia, artificial liver treatment

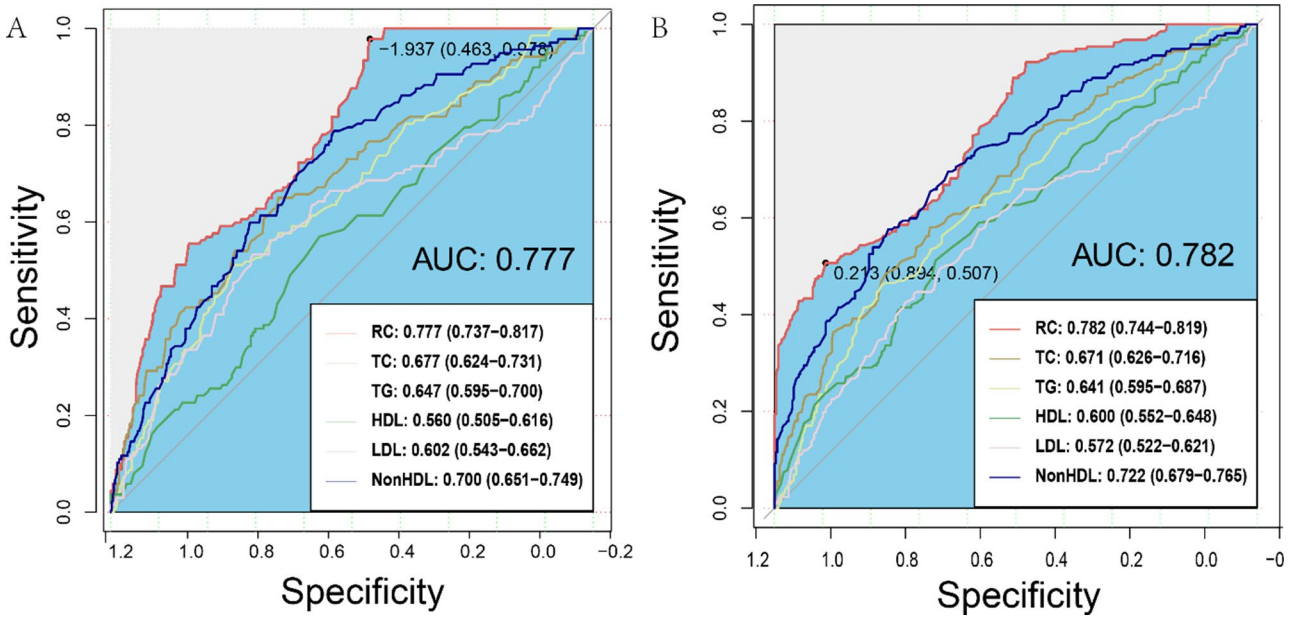

Fig. 3 Predictive capabilities of lipid parameters for poorer hepatitis B-related acute-on-chronic liver failure (HBV-ACLF). Receiver operating characteristic curves for the lipid parameters remnant cholesterol (RC), total cholesterol (TC), triglycerides (TG), high- (HDL-c) and low-density lipoprotein cholesterol (LDL-c), as well as Non-HDL-c, in terms of predicting poorer **A** 28- and **B** 90-day HBV-ACLF prognoses

Discussion

Lipids, aside from their role in energy metabolism, are also involved in regulating cell signal transduction, serving as key anti- and inflammatory molecules [16–17]. The liver is an indispensable organ, participating in several stages of lipoprotein and lipid synthesis, metabolism and secretion [18]. Additionally, lipid indicators have been found in a number of studies to serve as potential predictors for ACLF occurrence and mortality, such as lysophosphatidylcholine and cholesterol ester for early prediction of HBV-related ACLF, as well as 6-month mortality [19–20].

With respect to RC, it is rich in TG and lipoprotein cholesterol, which is composed of chylomicron remnants, as well as intermediate- and very-low-density lipoproteins [21]. It has been associated with multiple atherogenic effects, including monocyte activation, upregulation of proinflammatory cytokines, and increased thrombotic factors [22]. Consequently, high RC levels has also been found in previous studies to be associated with increased hypertension, diabetes, and cardiovascular disease risks [23–26]. Additionally, RC has been connected with liver disease, particularly metabolic dysfunction-associated fatty liver disease (MAFLD), as demonstrated by Wang et al., who observed that RC levels were positively correlated with higher homeostasis model assessment for insulin resistance scores, a diagnostic indicator of MAFLD, among MAFLD patients, indicating that elevated RC may be an independent risk factor for the disease [27]. These associations between liver diseases and higher RC levels are further reinforced by the findings of this study, which, based on the current literature, is the first to demonstrate a correlation between elevated RC levels with poorer 28- and 90-day HBV-ACLF prognoses. After dividing 595 HBV-ACLF patients into 3 groups, based on RC tertiles, it was observed that higher RC levels were associated with worse liver function, coagulation, and ACLF prognosis under restricted cubic spline and Kaplan-Meier analyses; furthermore, the positive relationship between higher RC and adverse ACLF prognosis risk was still present, even after excluding all other confounding factors, under multivariate Cox regression analyses. Under stratification analysis, though, an association between RC, hypoproteinemia, and poorer ACLF prognoses, in which patients with hypoproteinemia had worse ACLF prognoses than those who did not, which could be owed to hypoproteinemia stemming from lowered lipoprotein synthesis and increased degradation, resulting in lowered blood protein levels.

In terms of 28- and 90-day HBV-ACLF prognoses, ROC curve analyses identified that RC was more strongly predictive for adverse prognoses, compared to other lipid indicators, such as HDL-c, TC, TG, LDL-c, and non-HDL-c. On the other hand, those other indicators have been found in previous studies to be connected to liver function and disease. For instance, TC, being mainly synthesized by hepatocytes, is considered to be able, to some extent, reflect the synthetic capacity of the liver, as well as being a predictor for the nutritional status of a patient [28]. With respect to LDL-c and HDL-c, they are both considered to correlate with ACLF prognoses and survival, such as Xiao et al. showing that decreases in LDL-c levels could serve as an independent risk factor for HBV-related ACLF patient survival [29], as well as Kawamoto et al. [30] observing that LDL-c recovery towards healthy levels reflect liver functional recovery in the early stages of liver regeneration post-hepatectomy. These studies thus demonstrate that LDL-c levels could be a valuable marker for determining liver disease prognoses, particularly for ACLF, which may be owed to ACLF having lowered TC, and subsequently less endogenous cholesterol being carried by LDL, leading to decreased LDL-c. Another factor behind lowered LDL-c being linked to ACLF may be due to damages in phosphatidylcholine cholesterol acyltransferase [31]. As for HDL-c, it has been considered to play an important role in reducing inflammatory responses, which may be related to it being able to regulate cell membrane cholesterol levels, by removing cholesterol and other lipids from cells, as well as lowering inflammatory receptor signaling via Toll-like receptor signaling [32]. Moreover, HDL-c reduces the inflammatory response of liver disease patients by neutralizing endotoxins, such as bacterial lipopolysaccharide, and promoting excretion of the resulting products [33]. In terms of liver disease prognoses, HDL-c has had a mixed picture, with He et al. [34] observing that it was an independent predictor of poor short-term prognoses for HBV-related decompensated cirrhosis, while for ACLF, Wen et al. [35] proved that it had good prognostic value for predicting 1-year survival, but not for 90-day prognoses. In light of previous investigations, the observation that RC was the most predictive for poorer 28- and 90-day ACLF prognoses, compared to other lipid parameters, remediates the deficiency of those parameters, such as HDL-c being unable to accurately predict 90-day ACLF prognoses. RC is consequently an easily measurable potential parameter that could be used to accurately assess ACLF prognoses, in turn aiding the development of effective treatment plans for the disease.

The underlying bases behind the association of higher RC levels with poorer ACLF prognoses could be owed to multiple factors, one of which is that RC is linked to increased reactive oxygen species and pro-inflammatory cytokine production, leading to endothelial dysfunction, as well as exacerbated liver damage and inflammation in ACLF patients [36]. This increased endothelial dysfunction is also owed to increased RC contributing to increased atherosclerosis, which stems from RC being comprised of triglyceride-rich lipoproteins, particularly intermediate and low-density lipoproteins, as well as chylomicron remnants, as noted by Castañer et al. [6]. These lipoproteins are larger than LDL-c particles, rendering them more liable to be trapped within the intima [6]. This subsequently leads to increased recruitment of macrophages, which produce lipoprotein lipase to degrade the triglycerides and phagocytose the degradation products to become foam cells [6]. Higher foam cell numbers, in turn, yields greater atherosclerotic plaque formation [37]. Furthermore, Langsted et al., found that triglyceride-rich lipoproteins, due to their greater abundance and

larger sizes, are enriched with cholesterol as they circulate, resulting in them possessing larger amounts than that of LDL-c [37]. This cholesterol, unlike triglycerides, are unable to be degraded by cells, leading to their accumulation within the intima, and contributing to atherosclerotic plaque formation [37]. Consequently, these processes could serve as the basis behind the observation from Jepsen et al. that increased RC concentrations were associated with higher ischemic heart disease mortality, which was not found for higher LDL-c [38]. Additionally, the exacerbation in liver damage lowers the capability of the liver to remove triglyceride-rich lipoproteins, thereby yielding RC accumulation, which further contributes to increased inflammatory and oxidative stress burdens [36]. Ultimately, the 3 plausible underlying mechanisms behind higher RC being associated with poorer ACLF prognoses are via increased inflammation and oxidative stress, plus pro-atherogenic processes and impaired lipoprotein clearance; all these processes mutually reinforce each other in a vicious cycle to worsen ACLF prognoses.

Study strengths and limitations

The strengths of this study are that, for the first time, it explored the relationship between RC and HBV-ACLF prognoses. Moreover, its findings, based on a large sample size and applying strict statistical adjustments, can be considered to be relatively objective. Additionally, RC values are easily calculated and obtained, which are highly conductive for assessing HBV-ACLF risks, as well as carrying out epidemiological surveys in the general population.

However, there are a number of limitations in this study, one of it being its retrospective nature, which may reduce the predictive value of the results. Another limitation is that the 595 patients recruited for this study all came from a single center, meaning that the population may not be fully reflective of all HBV-ACLF patient demographics, and thus the observations may not be fully applicable for other HBV-ACLF groups. Therefore, future studies will involve multiple centers, with larger patient sample sizes that are more representative of overall HBV-ACLF patient demographics; an external validation dataset will also be established to confirm the robustness of the findings. Moreover, this study only looked at 28- and 90-day prognoses, which is due to the fact that after 90 days, little change in HBV-ACLF mortality rates are present, as demonstrated by Xiao et al., in which 90-day HBV-ACLF survival was 48.8%, and only slightly decreased afterwards, with 1-, 5-, and 8-year survival rates being, respectively, 46.1%, 43.8%, and 42.2% [39]. This indicates that HBV-ACLF individuals who survived after 90 days are still likely to be alive afterwards, for up to 8 years. Nevertheless, future studies will examine the associations between RC levels and longer-term prognoses of HBV-ACLF patients, such as extended mortality or complications. Additionally, even though efforts have been made to limit the influence of potential confounding variables, some other variables, such as in terms of dietary habits or genetic predispositions that could affect RC levels and ACLF disease progression, may have been overlooked. This could result in confounding biases still being present in the findings of this study, limiting their interpretative value [40]. Yet another limitation is that the potential for specific treatment or lifestyle changes, such as lipid-lowering therapies, to reduce RC levels could affect ACLF prognoses. It has been suggested, though, by Castañer et al. that RC levels could be lowered by statins, PCSK9 inhibitors, as well as RNA-based antisense oligonucleotide inhibitors of apolipoprotein C-III and angiopoietin-like 3 genes [6]. However, these potential RC-lowering agents have not been fully investigated in randomized clinical trials; thus, such trials should be carried out in future studies to identify their effectiveness. Lastly, the laboratory parameters in this study were measured only at the time of admission. Consequently, future studies should measure these parameters from the time of initial hospitalization to the end of the follow-up period, which would reflect the fluctuations in those parameters during the course of hospitalization.

Conclusion

Higher RC levels were found to be inversely associated with worse HBV-ACLF prognoses, particularly in the form of lower 28- and 90-day HBV-ACLF survival rates. Furthermore, the correlation between higher RC and worse HBV-ACLF prognoses, as well as worse liver function and coagulation, was still present even after excluding all other potential confounding factors. In particular, those with higher RC combined with hypoproteinemia were significantly more likely to have worse HBV-ACLF prognoses. RC was also the most accurate lipid parameter for predicting poorer 28- and 90-day ACLF prognoses, compared to TC, TG, HDL-c, LDL-c, and Non-HDL-c. Therefore, monitoring RC, along with developing interventions to reduce their levels, could aid in improving survival likelihoods among HBV-ACLF patients.

Abbreviations

ACLF	Acute-on-chronic liver failure
ALB	Albumin
ALT	Alanine aminotransferase
ANOVA	One-way analysis of variance
AST	Aspartate aminotransferase
AUC	Area under the curve
Bil	Bilirubin
BUN	Blood urea nitrogen
CI	Confidence interval
DBIL	Direct bilirubin
FPG	Fasting plasma glucose
GGT	γ-glutamyl transferase

HBV-ACLF	Hepatitis B-related acute-on-chronic liver failure
HDL-c	High-density lipoprotein cholesterol
HR	Hazard ratio
LDL-c	Low-density lipoprotein cholesterol
MAFLD	Metabolic dysfunction-associated fatty liver disease
NAFLD	Non-alcoholic fatty liver disease
PLT	Platelets
PT	Prothrombin time
RC	Remnant cholesterol
ROC	Receiver operating characteristic
TBil	Total bilirubin
TC	Total cholesterol
TG	Triglycerides
WBC	White blood cells
RCS	Restricted cubic spline

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Author contributions
JL conceived the idea for the study and was a major contributor in writing the manuscript. JL and WZ managed the logistics for this study and was a major contributor in revising the manuscript. SY and JF supervised the study. YW was a major contributor in revising the manuscript. WZ conceived the idea for the study, designed and supervised the study, and provided all final approvals for all stages of the study.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval consent to participate
The study was carried out according to the Declaration of Helsinki and was approved by the medical ethics committee of the First Affiliated Hospital of Nanchang University in China (no. IIT-2022-096). All patients provided written informed consent to participate in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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