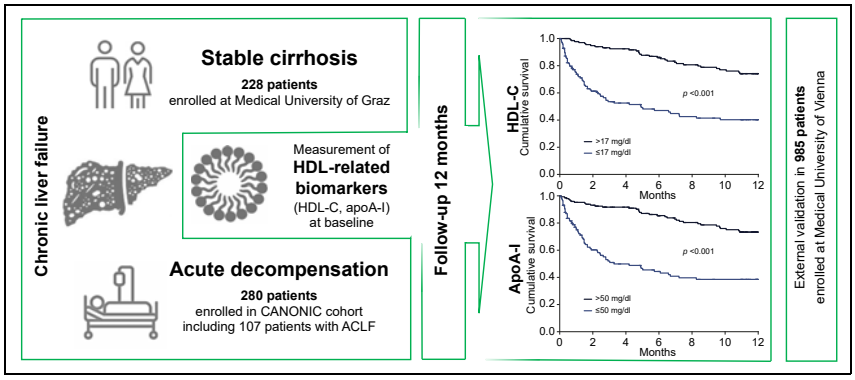
Research Article
Cirrhosis and Liver Failure

JOURNAL
OF HEPATOLOGY

HDL-related biomarkers are robust predictors of survival in patients with chronic liver failure

Graphical abstract



Highlights

- HDL levels are profoundly decreased in chronic liver failure.
- HDL-related biomarkers (HDL-C, apoA-I) are robust predictors of disease progression and survival.
- The prognostic value of single HDL-related biomarkers is very similar to that of the composite scores.
- HDL-related biomarkers correlated inversely with markers of inflammation.

Authors

Markus Trieb, Florian Rainer, Vanessa Stadlbauer, …, Joan Clària, Gunther Marsche, Rudolf E. Stauber

Correspondence

gunther.marsche@medunigraz.at (G. Marsche), rudolf.stauber@medunigraz.at (R.E. Stauber).

Lay summary

People who suffer from cirrhosis (scarring of the liver) have low levels of cholesterol carried by high-density lipoproteins (HDL-C). These alterations are connected to inflammation, which is a problem in severe liver disease. Herein, we show that reduced levels of HDL-C and apolipoprotein A-I (apoA-I, the main protein carried by HDL) are closely linked to the severity of liver failure, its complications and survival. Both HDL-C and apoA-I can be easily measured in clinical laboratories and are as good as currently used prognostic scores calculated from several laboratory values by complex formulas.

https://doi.org/10.1016/j.jhep.2020.01.026
 J. Hepatol. 2020, 73, 113–120

Research Article
Cirrhosis and Liver Failure

JOURNAL OF HEPATOLOGY

HDL-related biomarkers are robust predictors of survival in patients with chronic liver failure

Markus Trieb[1,2,#], Florian Rainer[3,#], Vanessa Stadlbauer[3], Philipp Douschan[4], Angela Horvath[3,5], Lukas Binder[3], Athina Trakaki[1], Eva Knuplez[1], Hubert Scharnagl[6], Tatjana Stojakovic[7], Ákos Heinemann[1], Mattias Mandorfer[8,9], Rafael Paternostro[8,9], Thomas Reiberger[8,9], Carla Pitarch[10], Alex Amorós[10], Alexander Gerbes[11], Paolo Caraceni[10,12], Carlo Alessandria[10,13], Richard Moreau[10,14], Joan Clària[10,15], Gunther Marsche[1,2,*], Rudolf E. Stauber[3,*]

[1]Division of Pharmacology, Otto Loewi Research Center, Medical University of Graz, Graz, Austria; [2]BioTechMed-Graz, Graz, Austria; [3]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Graz, Austria; [4]Division of Pulmonology, Department of Internal Medicine, Medical University of Graz, Graz, Austria; [5]Center for Biomarker Research in Medicine (CBmed), Graz, Austria; [6]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; [7]Clinical Institute of Medical and Chemical Laboratory Diagnostics, University Hospital Graz, Graz, Austria; [8]Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [9]Vienna Hepatic Hemodynamic Lab, Medical University of Vienna, Vienna, Austria; [10]European Foundation for the Study of Chronic Liver Failure (EF Clif), Barcelona, Spain; [11]Department of Medicine II, University Hospital, LMU Munich, Munich, Germany; [12]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; [13]Division of Gastroenterology and Hepatology, Città della Salute e della Scienza Hospital, University of Turin, Turin, Italy; [14]Institut national de la Santé et de la Recherche Médicale, Université de Paris, Centre de Recherche sur l'Inflammation, Paris et Service d'hépatologie, Hôpital Beaujon, APHP, Clichy, France; [15]Hospital Clínic, IDIBAPS and CIBERehd, Barcelona, Spain

Background & Aims: High-density lipoprotein cholesterol (HDL-C) levels are reduced in patients with chronic liver disease and inversely correlate with disease severity. During acute conditions such as sepsis, HDL-C levels decrease rapidly and HDL particles undergo profound changes in their composition and function. We aimed to determine whether indices of HDL quantity and quality associate with progression and survival in patients with advanced liver disease.

Methods: HDL-related biomarkers were studied in 508 patients with compensated or decompensated cirrhosis (including acute-on-chronic liver failure [ACLF]) and 40 age- and gender-matched controls. Specifically, we studied levels of HDL-C, its subclasses HDL2-C and HDL3-C, and apolipoprotein A1 (apoA-I), as well as HDL cholesterol efflux capacity as a metric of HDL functionality.

Results: Baseline levels of HDL-C and apoA-I were significantly lower in patients with stable cirrhosis compared to controls and were further decreased in patients with acute decompensation (AD) and ACLF. In stable cirrhosis (n = 228), both HDL-C and apoA-I predicted the development of liver-related complications independently of model for end-stage liver disease (MELD) score. In patients with AD, with or without ACLF (n = 280), both HDL-C and apoA-I were MELD-independent predictors of 90-day mortality. On ROC analysis, both HDL-C and apoA-I had high diagnostic accuracy for 90-day mortality in patients with AD (AUROCs of 0.79 and 0.80, respectively, similar to that of MELD 0.81). On Kaplan-Meier analysis, HDL-C <17 mg/dl and apoA-I <50 mg/dl indicated poor short-term survival. The prognostic accuracy of HDL-C was validated in a large external validation cohort of 985 patients with portal hypertension due to advanced chronic liver disease (AUROCs HDL-C: 0.81 *vs.* MELD: 0.77).

Conclusion: HDL-related biomarkers are robust predictors of disease progression and survival in chronic liver failure.

Lay summary: People who suffer from cirrhosis (scarring of the liver) have low levels of cholesterol carried by high-density lipoproteins (HDL-C). These alterations are connected to inflammation, which is a problem in severe liver disease. Herein, we show that reduced levels of HDL-C and apolipoprotein A-I (apoA-I, the main protein carried by HDL) are closely linked to the severity of liver failure, its complications and survival. Both HDL-C and apoA-I can be easily measured in clinical laboratories and are as good as currently used prognostic scores calculated from several laboratory values by complex formulas.

Keywords: HDL; apoA-I; MELD score; ACLF; Inflammation.
Received 16 August 2019; received in revised form 21 January 2020; accepted 31 January 2020; available online 14 February 2020

* Corresponding authors. Addresses: Division of Pharmacology, Otto Loewi Research Center, Medical University of Graz, Universitätsplatz 4, 8010 Graz, Austria; Tel.: +43 316 385 74128, fax: +43 316 385 79613 (G. Marsche), or Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria; Tel.: +43 316 385 80268, fax: +43 316 385 17108 (R.E. Stauber).
E-mail addresses: gunther.marsche@medunigraz.at (G. Marsche), rudolf.stauber@medunigraz.at (R.E. Stauber).
# Markus Trieb and Florian Rainer contributed equally to this work.
https://doi.org/10.1016/j.jhep.2020.01.026

Introduction

Chronic liver disease is a growing public health problem across Europe and the United States.[1,2] Since the liver plays an essential role in several phases of lipid synthesis, transport, and metabolism, hypocholesterolemia often occurs in patients with cirrhosis.[3] Cirrhosis is associated with a decrease in serum levels of high-density lipoprotein cholesterol (HDL-C), low-density

lipoprotein[4] and apolipoprotein (apo) A-I, the main protein component of HDL particles.[5] Lipoprotein levels further decrease with increasing severity of liver disease.[6,7] Moreover, low HDL-C and apoA-I levels significantly associate with death or need for liver transplantation in patients with non-cholestatic cirrhosis[8] and associate with the severity of septic disease and an exaggerated systemic inflammatory response.[9] This is consistent with the ability of HDL to bind and neutralize bacterial lipopolysaccharide (LPS) and to promote excretion of these products,[10,11] raising the possibility that HDL particles play a critical role in the pathophysiology of chronic liver failure. HDL consists of 2 large subclasses, namely large, buoyant HDL2 particles and small, dense HDL3 particles. Recent studies provided evidence that the anti-inflammatory properties of HDL3 are superior to those of HDL2.[12] HDL3 effectively mobilizes cholesterol from cholesterol-rich microdomains, which serve as signal platforms for Toll-like receptors and thus attenuate the inflammatory response.[13] This may increase susceptibility of patients with cirrhosis to bacterial infections, which are the most common triggers of acute-on-chronic liver failure (ACLF).[14] We have recently shown that cirrhosis alters HDL composition and function and that HDL cholesterol efflux capacity (CEC) predicted 12-month mortality in patients with cirrhosis.[15] However, the measurement of CEC is very demanding and currently unsuitable as a routine clinical test. In the present study, we investigated the prognostic value of several HDL-related biomarkers that can be easily integrated into routine clinical testing, *i.e.* HDL-C, apoA-I, and the HDL subclasses HDL2-C and HDL3-C in a large cohort of patients with compensated and decompensated advanced chronic liver disease (ACLD), including ACLF. In addition, we assessed CEC as a metric of HDL function.

Patients and methods

Patients

The patient population of this explorative cross-sectional study included 508 patients with cirrhosis from 2 different cohorts: (i) 228 consecutive patients with stable cirrhosis recruited between 2011 and 2016 at the Medical University of Graz (outpatient clinic or gastrointestinal ward including stable patients undergoing evaluation for liver transplantation) and (ii) 280 patients with decompensated cirrhosis with or without ACLF who were investigated between February and September 2011 in the multicenter CANONIC study in 12 European countries (for further details, see Moreau *et al.*[16]). In both studies, the diagnosis of cirrhosis was based on liver histology or a combination of clinical, biochemical and imaging signs. Patients with prior solid organ transplantation or the presence of hepatocellular carcinoma were excluded. Due to the impact of cholestasis on lipid profiles, patients with cholestatic liver diseases were excluded.[17] Hospitalized patients with cirrhosis were examined for the presence of acute decompensation (AD) or ACLF as defined by Moreau *et al.*[16] Besides baseline data (history, physical examination, and laboratory measurements), information on liver transplantation and mortality at 90 days and 12 months following enrollment were recorded. In addition, data regarding the occurrence of complications of cirrhosis were obtained in patients with stable cirrhosis at baseline. Complications of cirrhosis were defined as follows: development of new-onset ascites in a patient without ascites at baseline, development of new-onset hepatic encephalopathy, development of spontaneous bacterial peritonitis, hepatorenal syndrome, new-onset jaundice (clinically detectable or bilirubin >3 mg/dl), upper gastrointestinal bleeding due to portal hypertension (variceal bleeding or bleeding from portal hypertensive gastropathy), occurrence of portal vein thrombosis, or death from liver disease. Besides, occurrence of severe infection requiring antibiotic therapy within 12 months was assessed in patients with stable cirrhosis.

Furthermore, 40 age- and sex-matched healthy controls who did not meet the following exclusion criteria were included: any history of cardiovascular disease, pregnancy, obesity, dyslipidemia, liver disease, renal disease, diabetes or clinical signs of inflammation. Control participants did not take lipid-lowering medication or anti-inflammatory drugs. Blood was sampled at baseline from patients and healthy controls.

In addition, HDL-C was investigated in an external validation cohort composed of 985 consecutive patients who underwent hepatic venous pressure gradient (HVPG) measurement between 2004 and 2018 at the Vienna Hepatic Hemodynamic Lab at the Medical University of Vienna ("HVPG cohort"). Patients with a normal HVPG (≤5 mm Hg), porto-sinusoidal vascular disease, cholestatic liver disease, transjugular intrahepatic portosystemic shunt, a history of liver transplantation, or missing information on HDL-C were excluded.

All 3 studies had been approved by the local Institutional Review Boards (IRBs) in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient unless the requirement had been waived by the local IRB.

Lipid and cytokine assays

Levels of total cholesterol were measured enzymatically (Diasys, Holzheim, Germany). The levels of total HDL-C and HDL3-C were determined using homogeneous assays from Denka Seiken Co., Ltd. (Tokyo, Japan).[18] HDL2-C was estimated by subtracting HDL3-C from total HDL-C. ApoA-I was determined by immunoturbidimetry as described.[19] Analyses were performed on an Olympus AU680 analyzer (Beckman Coulter, Brea, USA). Cytokines (interleukin [IL]-6, IL-8, tumor necrosis factor-α [TNF-α]) were quantified using a multiplexed bead-based immunoassay.

Preparation of apoB-depleted serum

ApoB-depleted serum was prepared by addition of 40 μl of polyethyleneglycol (20% in 200 mmol/L glycine buffer) to 100 μl of serum.[15] Serum was incubated at room temperature for 20 min and the supernatant recovered after centrifugation (10,000 rpm, 20 min, 4°C).

HDL cholesterol efflux capacity

CEC was assessed using an established assay.[20,21] J774 macrophages, maintained in DMEM with 10% fetal bovine serum were plated on 48-well plates (300,000 cells/well). Cells were labeled for 24 h with 1 μCi/ml [^{3}H]-cholesterol (Perkin Elmer, Boston, MA, USA). [^{3}H]-cholesterol-labeled J774 macrophages were incubated in the presence of 0.3 mmol/L 8-(4-chlorophenylthio)-cAMP to stimulate ATP-binding cassette transporter A1 (ABCA1) expression. After labeling, cells were washed twice with serum-free DMEM and subsequently equilibrated in serum-free DMEM containing 0.2% BSA for 2 h. After 2 additional washing steps, [^{3}H]-cholesterol efflux was determined by incubating cells for 3 h with serum-free DMEM containing 2.8% apoB-depleted serum. Cholesterol efflux was expressed as the radioactivity in the medium relative to total radioactivity in medium and cells. A control

serum was used on each plate to decrease inter-plate variability. All steps were performed in the presence of 2 μg/ml of the acyl coenzyme A cholesterol acyltransferase inhibitor Sandoz 58-035 (Sigma, Darmstadt, Germany).

Composite prognostic scores

The prognostic value of HDL-related biomarkers (as single prognostic parameters) was compared with established composite prognostic scores as appropriate: (i) with the model for end-stage liver disease (MELD) score in all patient groups, (ii) with the Chronic Liver Failure Consortium (CLIF-C) AD score[22] in patients with AD, and (iii) with CLIF-C ACLF score[23] in patients with ACLF.

Statistical analysis

Continuous variables are reported by median (Q1–Q3) whereas categorical data are presented as frequencies and percentages. Non-normally distributed variables were compared non-parametrically with Kruskal-Wallis tests using Bonferroni correction for multiple comparisons. Correlations were analyzed by Pearson product-moment estimates. Statistical analyses were performed using GraphPad Prism (Version 4.0, GraphPad Software) or SPSS Statistics Version 26 (SPSS Inc., Chicago, IL, USA). Univariate binary logistic regression analysis was used to evaluate prognostic variables for development of complications. Odds ratios estimated from logistic regression are reported with corresponding 95% CIs. Nagelkerke's Pseudo-R^2 is given for each model as a measure of goodness-of-fit. Variables identified as potential covariates (p <0.10) from univariate analyses were then tested in multivariable logistic regression models. Competing risk analysis was performed to assess the prognostic value of HDL-related biomarkers for 90-day and 12-month mortality. Either HDL or apoA-I was added to MELD and analyzed by multivariable competing risk analysis with death as the event and liver transplantation as a competing risk, using SAS version 9.4. In addition, the performance of prognostic variables was tested by ROC analysis. AUROCs were compared by the method of DeLong *et al.*[24] using MedCalc version 14.10.2 (MedCalc Software, Ostend, Belgium). The effect of prognostic variables on mortality was analyzed by the Kaplan-Meier method and compared by log-rank test. The results of the study are reported following the TRIPOD (transparent reporting of a multivariable prediction model for individual prognosis or diagnosis) statement recommendations.[25]

Results

Patient characteristics

In total, 508 patients were enrolled in 2 cohorts, 228 patients with stable cirrhosis in the Austrian single-center and 280 patients with AD in the European multicenter cohort. Among patients with stable cirrhosis, 131 patients (57.5%) were classified as Child-Pugh class A, 74 patients (32.5%) Child-Pugh class B and 23 patients (10%) class C. Among the 280 patients with AD, 107 were diagnosed with ACLF. Further details on characteristics of patients (including mortality data) and of controls are given in Table 1. Patients who were transplanted during follow-up were censored for ROC analyses. Nine patients with stable cirrhosis (4%) were taking lipid-lowering drugs while this information was not available for patients with AD. Due to the small number of cases in the stable cirrhosis cohort and presumably low lipid-lowering medication in patients with AD, no further analyses with respect to lipid-lowering medication were performed. Patient characteristics of the external validation cohort from Vienna (HVPG cohort) are given in Table S1.

HDL-related biomarkers correlate with liver disease severity

We assessed HDL-C and apoA-I in all 508 patients and 40 age- and gender-matched controls. Furthermore, the major HDL subclasses HDL2-C and HDL3-C as well as CEC were determined in a subset of 333 patients (228 patients with stable cirrhosis and 105 patients with AD). Median levels of total cholesterol and HDL-related biomarkers, including HDL-C, HDL3-C, apoA-I, and CEC, were significantly lower in patients with cirrhosis compared to healthy controls and gradually decreased with increasing severity of disease (Table 2, Fig. 1).

Table 1. Characteristics of study participants.

	Controls	Stable cirrhosis	AD	ACLF
N	40	228	173	107
Age [years]	57 (48–64)	58 (51–63)	58 (51–67)	55 (48–64)
Gender (% male)	60%	75%	64%	64%
Etiology of cirrhosis				
Alcohol		58%	53%	57%
HCV		17%	31%	28%
Other		25%	16%	15%
Child-Pugh score		6 (5–8)	9 (8–11)	11 (10–13)
MELD		12 (9–16)	16 (12–20)	27 (22–33)
CLIF-C AD score		n.a.	52 (46–57)	n.a.
CLIF-C ACLF score		n.a.	n.a.	49 (43–56)
WBC [10^9/L]	6.0 (5.1–6.7)	5.1 (3.9–6.5)	5.7 (4.1–8.2)	8.4 (5.3–12.3)
PLT [10^9/L]	249 (218–270)	111 (79–150)	97 (59–141)	67 (42–114)
Bilirubin [mg/dl]	0.5 (0.4–0.7)	1.4 (0.8–2.8)	2.8 (1.4–5.6)	7.4 (2.3–18.7)
Creatinine [mg/dl]	0.8 (0.7–0.9)	0.8 (0.7–1.0)	0.9 (0.7–1.2)	1.9 (0.9–3.2)
INR	1.01 (0.98–1.04)	1.28 (1.16–1.48)	1.46 (1.27–1.75)	1.88 (1.40–2.52)
Sodium [mmol/L]	140 (139–141)	139 (136–141)	136 (133–139)	134 (128–137)
Albumin [g/dl]	4.4 (4.3–4.6)	4.0 (3.3–4.4)	2.9 (2.6–3.2)	3.0 (2.3–3.4)
90-day mortality		4%	15%	42%
12-month mortality		7%	27%	56%

ACLF, acute-on-chronic liver failure; AD, acute decompensation; CLIF-C, Chronic Liver Failure Consortium; INR, international normalized ratio; MELD, model for end-stage liver disease; PLT, platelet count; WBC, white blood cell count. Data are shown as median (Q1–Q3). n.a., not applicable.

Table 2. HDL-related biomarkers.

	Controls	Stable cirrhosis	AD	ACLF
N	40	228	173	107
Cholesterol [mg/dl]	231 (199–285)	149 (120–181)	95 (73–126)	71 (42–100)
HDL-C [mg/dl]	63 (52–83)	47 (36–60)	22 (11–30)	11 (5–20)
HDL2-C [mg/dl]	30 (23–48)	28 (19–38)	13 (7–20)	5 (0–10)
HDL3-C [mg/dl]	32 (27–41)	18 (13–23)	11 (7–15)	6 (4–10)
ApoA-I [mg/dl]	178 (152–210)	122 (90–149)	69 (43–89)	33 (18–67)
CEC	12.2 (10.6–14.3)	9.7 (7.3–11.9)	5.8 (4.4–7.6)	4.6 (3.8–7.2)

AD, acute decompensation; ACLF, acute-on-chronic liver failure; apoA-I, apolipoprotein A1; CEC, cholesterol efflux capacity; HDL-C, high-density lipoprotein cholesterol. Data are shown as median (Q1–Q3).

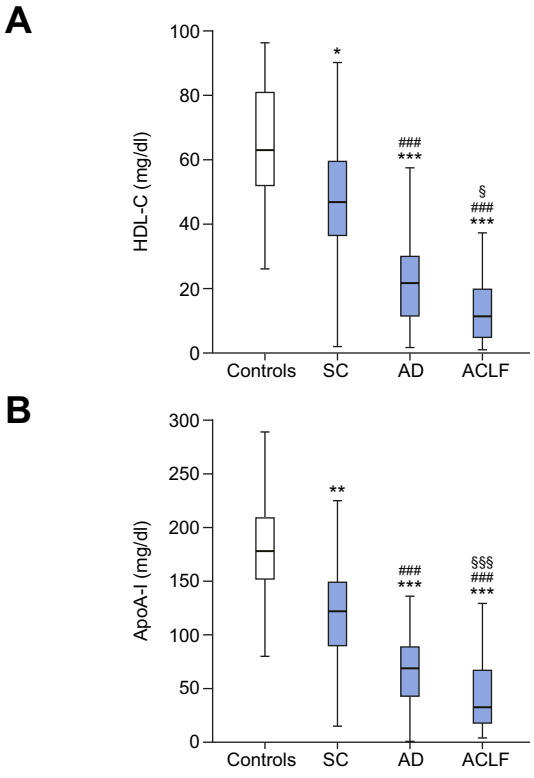


Fig. 1. HDL-related biomarkers are associated with severity of chronic liver failure. Plasma samples of healthy controls (n = 40) and patients with SC (n = 228), AD (n = 173) and ACLF (n = 107) were examined for levels of (A) HDL-C and (B) apoA-I. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 *vs.* control; $^{\#\#\#}p$ <0.001 *vs.* SC; $^{\S}p$ <0.05, $^{\S\S\S}p$ <0.001 *vs.* AD (Kruskal-Wallis test with Bonferroni correction). ACLF, acute-on-chronic liver failure; AD, acute decompensation; ApoA-I, apolipoprotein A1; HDL-C, high-density lipoprotein cholesterol; SC, stable cirrhosis.

HDL-related biomarkers correlate with inflammatory markers

The pathophysiology of chronic liver failure is determined by several factors, including systemic inflammation.[26,27] Therefore, we determined the concentrations of various inflammatory markers in subsets of our cohort of patients with cirrhosis. Compared to patients without ACLF, patients with ACLF showed significantly higher numbers of white blood cells (Fig. S1A) and higher C-reactive protein (CRP) levels (Fig. S1B), IL-6 (Fig. S1C) and TNF-α (Fig. S1E), while IL-8 remained unchanged (Fig. S1D). Levels of CRP increased progressively with stage of cirrhosis (Fig. S1B). Since HDL shows anti-inflammatory activity, we next investigated whether HDL-related biomarkers are associated with indices or mediators of inflammation in these subsets.

Table 3. Independent predictive factors with respect to development of complications in stable cirrhosis (n = 228).

	HR	95% CI	*p* value	R^2
Univariate				
HDL-C	0.96	0.94–0.98	<0.001	0.163
ApoA-I	0.97	0.96–0.98	<0.001	0.315
MELD	1.30	1.19–1.41	<0.001	0.334
Add to MELD				
HDL-C	0.98	0.96–1.00	0.061	
MELD	1.26	1.15–1.37	<0.001	
Combined				0.346
ApoA-I	0.98	0.97–0.99	0.001	
MELD	1.19	1.08–1.31	<0.001	
Combined				0.387

Multivariable logistic regression. ApoA-I, apolipoprotein A1; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; MELD, model for end-stage liver disease; R^2, Nagelkerke's R^2.

Interestingly, we found strong negative associations of total cholesterol and all HDL-related biomarkers (HDL-C, HDL2-C, HDL3-C, apoA-I, and CEC) with white blood cell count, CRP, IL-6, IL-8, and TNF-α (Table S2).

HDL-related biomarkers predict complications and mortality

Complications of cirrhosis and mortality in patients with stable cirrhosis

The predictive values of HDL-related biomarkers regarding occurrence of complications within 12 months were assessed in patients with stable cirrhosis at baseline (n = 228). 27 patients had to be excluded due to insufficient follow-up (n = 8) or insufficient data with respect to complications (n = 19). In total, 201 patients were included for analysis. Overall, complications occurred in 77 of 201 patients (38%); usually more than 1 complication per patient was recorded (Table S3). In univariate analysis, total cholesterol, HDL-C, HDL2-C, HDL3-C, apoA-I, CEC, international normalized ratio, bilirubin, Child-Pugh and MELD scores were associated with development of complications (data not shown). In order to avoid multicollinearity, only 1 lipid-related biomarker and MELD score were included in multivariate analyses. Multivariable logistic regression yielded both HDL-C and apoA-I as independent predictors of complications (Table 3). On ROC analysis, HDL-C and apoA-I showed high diagnostic accuracy for 12-month mortality (Fig. 2A) and for development of complications within 12 months (Fig. 2B), with AUROCs very similar to those of MELD. Interestingly, HDL-C and apoA-I levels were significantly lower in patients who developed severe infections requiring antibiotic therapy within 12 months (HDL-C: 30 (20–48) mg/dl *vs.* 52 (42–65) mg/dl, p <0.001; apoA-I: 75 (49–114) mg/dl *vs.* 132 (107–153) mg/dl, p <0.001).

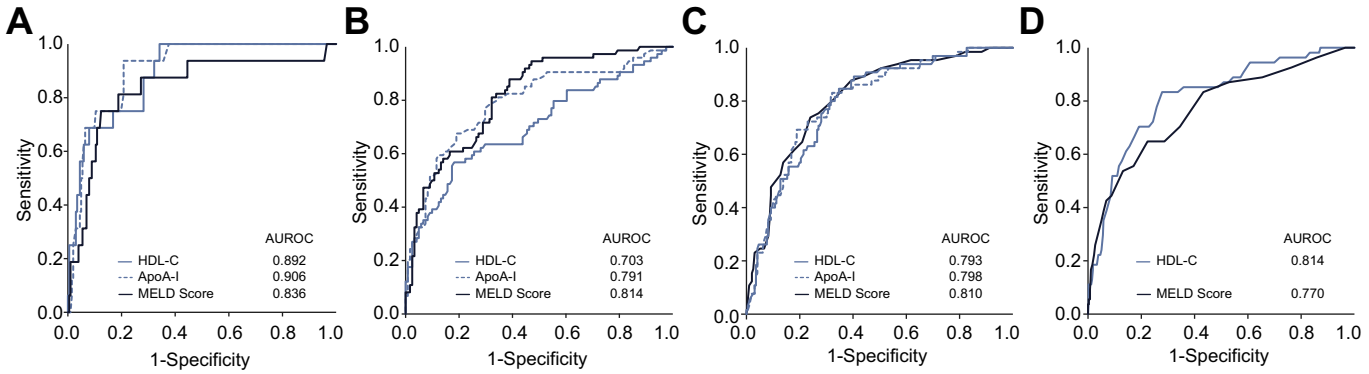


Fig. 2. HDL-related biomarkers predict complications and mortality. ROC curves illustrating the prognostic value of HDL-C, apoA-I and MELD score. (A) 12-month mortality in stable cirrhosis; (B) development of complications within 12 months in stable cirrhosis; (C) 90-day mortality in AD/ACLF cohort; (D) 90-day mortality in external validation set (HVPG cohort). ACLF, acute-on-chronic liver failure; AD, acute decompensation; ApoA-I, apolipoprotein A1; HDL-C, high-density lipoprotein cholesterol; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease.

Table 4. Independent predictive factors of 90-day and 12-month mortality in CANONIC patients (AD+ACLF, n = 280).

	HR	95% CI	*p* value
	90-day mortality		
Univariate			
HDL-C	0.91	0.89–0.94	<0.001
ApoA-I	0.97	0.96–0.98	<0.001
Add to MELD			
HDL-C	0.94	0.92–0.97	<0.001
MELD	1.09	1.05–1.13	<0.001
ApoA-I	0.98	0.97–0.99	<0.001
MELD	1.08	1.04–1.12	<0.001
	12-month mortality		
Univariate			
HDL-C	0.94	0.93–0.96	<0.001
ApoA-I	0.98	0.97–0.99	<0.001
Add to MELD			
HDL-C	0.97	0.95–0.99	0.004
MELD	1.07	1.04–1.11	<0.001
ApoA-I	0.99	0.98–0.99	0.019
MELD	1.07	1.04–1.11	<0.001

Competing risk analysis with death as event and liver transplantation as competing risk. ACLF, acute-on-chronic liver failure; AD, acute decompensation; ApoA-I, apolipoprotein A1; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; MELD, model for end-stage liver disease.

Mortality in patients with AD

The impact of HDL-related biomarkers on mortality was assessed in patients with AD with or without ACLF (n = 280) using competing risk analysis. Both HDL-C and apoA-I showed high predictive value for the outcomes of interest, *i.e.* 90-day and 12-month mortality, on univariate analysis and remained independent prognostic variables on multivariable analysis when added to MELD (Table 4). On ROC analysis, HDL-related biomarkers showed high diagnostic accuracy for 90-day mortality (Fig. 2C). When our AD cohort was split into the first (n = 105) and second (n = 175) dataset of CANONIC patients, ROC analysis yielded very similar results (Fig. S2A-B). Furthermore, the prognostic value of HDL-related biomarkers was compared with the CLIF-C AD score for patients with AD and with the CLIF-C ACLF score for patients with ACLF, again with similar results for HDL-related biomarkers and the respective composite scores (Fig. S3A-B). Of note, in all ROC analyses diagnostic accuracies of HDL-C and apoA-I were very similar to that of MELD (Table 5). Calculation of Youden index identified optimal cut-offs of <17 mg/dl for HDL-C and of <50 mg/dl for apoA-I for prediction of 90-day mortality. On Kaplan-Meier analysis, baseline HDL-C or apoA-I values below these cut-offs were associated with markedly lower 90-day survival (Fig. 3A,B) and showed very similar prognostic value as a baseline MELD of ≥22 points (Fig. 3C).

Mortality in external validation cohort

In the external validation cohort from Vienna, including 985 patients with ACLD and portal hypertension (HVPG cohort), 54 patients died within 90 days. Again, HDL-C was an excellent predictor of 90-day mortality (AUROC HDL-C: 0.81 *vs.* MELD: 0.77, $p = 0.24$ by DeLong test) (Table 5, Fig. 2D).

Discussion

In this explorative cross-sectional study, we show that HDL-related biomarkers are robust predictors of disease progression and survival, respectively, in the setting of stable cirrhosis and/or AD. The prognostic values of HDL-C and apoA-I as single and readily available parameters were very similar to those of the composite scores MELD, CLIF-C AD and CLIF-C ACLF. Furthermore, the prognostic value of HDL-C was confirmed in a large external validation cohort of patients with ACLD and portal hypertension.

Several laboratory markers and functional scores have been studied as predictors of mortality in patients with cirrhosis. The Child-Pugh score comprises serum levels of bilirubin and albumin, prothrombin time, degree of ascites and severity of hepatic encephalopathy and was originally introduced to predict mortality in patients with cirrhosis undergoing surgery.[28] The MELD score was initially developed to predict mortality of patients undergoing transjugular intrahepatic portosystemic shunt insertions and employs serum levels of creatinine and bilirubin and the international normalized ratio.[29] We recently showed that HDL maturation, metabolism and function is significantly altered in patients with liver disease.[15] The results of the present study suggest that HDL-related biomarkers, such as HDL-C, HDL3-C, apoA-I and CEC, reflect deterioration in liver function and reliably predict disease progression, development of complications and survival in patients with cirrhosis. HDL-C and apoA-I can be easily measured in routine clinical laboratories and – as single parameters – show excellent diagnostic

Table 5. Predictive discrimination of 90-day mortality by HDL-related biomarkers compared to composite prognostic scores.

	AD/ACLF n = 280	AD n = 173	ACLF n = 107	HVPG cohort n = 985
	AUROC (95% CI)			
HDL-C	0.79 (0.73–0.85)	0.82 (0.74–0.89)	0.75 (0.64–0.85)	0.81 (0.76–0.87)
ApoA-I	0.80 (0.74–0.86)	0.82 (0.74 0.91)	0.73 (0.62–0.84)	
MELD	0.81 (0.75–0.87)	0.81 (0.70–0.91)	0.72 (0.61–0.83)	0.77 (0.70–0.84)
CLIF-C AD score		0.72 (0.62–0.82)		
CLIF-C ACLF score			0.75 (0.65–0.85)	

ACLF, acute-on-chronic liver failure; AD, acute decompensation; ApoA-I, apolipoprotein A1; CLIF-C, Chronic Liver Failure Consortium; HDL-C, high-density lipoprotein cholesterol; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease.

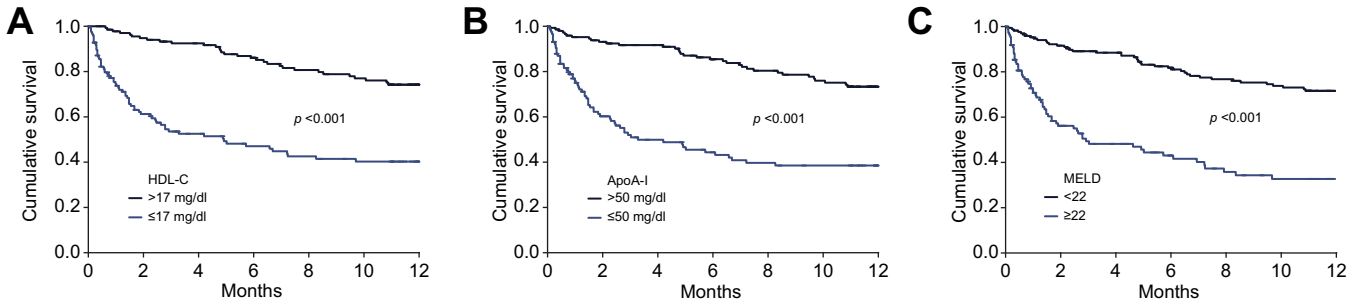


Fig. 3. Kaplan-Meier plots of survival probability in patients with acute decompensation of cirrhosis. (A) Mortality was significantly higher in patients with HDL-C <17 mg/dl (p <0.001 by log-rank test). (B) Mortality was significantly higher in patients with apoA-I <50 mg/dl (p <0.001 by log-rank test). (C) Mortality was significantly higher in patients with MELD ≥22 (p <0.001 by log-rank test). ApoA-I, apolipoprotein A1; HDL-C, high-density lipoprotein cholesterol; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease.

accuracies for 90-day and 12-month mortality which are very similar to those of composite scores such as Child-Pugh and MELD. In particular, HDL-C and apoA-I decrease with increasing severity of the disease regardless of the etiology of cirrhosis. Of note, baseline HDL-C <17 mg/dl and apoA-I <50 mg/dl are associated with dismal prognosis (90-day survival approx. 50%) and those prove useful as single biomarkers of short-term survival. Our findings are consistent with an earlier study by Poynard *et al.* that investigated 581 patients with alcohol-related liver disease and reported reduced 1-year survival of patients with cirrhosis and apoA-I <100 mg/dl.[7]

Bacterial infections are common in cirrhosis and account for significant mortality.[30] They are facilitated by immune dysfunction that worsens with liver disease progression and frequently results in ACLF.[31,32] The risk of individuals with cirrhosis becoming septic is more than doubled compared to the general population and their clinical outcomes are dismal despite intensive care.[33–35] HDL regulates the cholesterol levels of cell membranes through its ability to remove cholesterol and other lipid species from cells, thereby reducing inflammatory receptor signaling.[13,36] HDL particles bind LPS and reduce cytokine production and mortality in various animal models.[37–39] Accordingly, the susceptibility to LPS-induced liver damage is higher in rats with cirrhosis than in healthy animals[40] and decreases with HDL administration.[41,42]

In a previous study, we observed that cirrhosis significantly impaired the ability of HDL to suppress LPS-induced activation of the proinflammatory transcription factor NF-κB in monocytes and subsequent IL-6 and TNF-α production.[15] In the present study, we report a robust inverse association of HDL-related biomarkers with white blood cell count, CRP, IL-6, IL-8, and TNF-α. Furthermore, patients with subsequent infection showed significantly lower levels of HDL-related biomarkers. In the context of chronic liver failure, the immunomodulatory function of HDL is of particular interest. Reduced HDL quantity and function may play an important role in the pathophysiology of systemic inflammation that drives the development of ACLF.[27] In line with this concept, an *ex vivo* study in patients with advanced chronic liver failure has shown that the restoration of HDL function by addition of reconstituted HDL reduces the LPS-induced inflammatory response.[43] It should be noted that a similar therapeutic approach with reconstituted apoA-I (CSL112) infusions has already been attempted in patients with myocardial infarction and was found safe in a recent phase II study.[44]

This study has some limitations: our results, albeit robust, are observational, do not prove causality and may be subject to unmeasured confounders. Strengths of our study include the large number of patients (n = 508) representing the whole spectrum of chronic liver failure, separate analysis of individual outcome parameters in patients with stable cirrhosis *vs.* AD, long-term follow-up of the study cohort for at least 1 year, the detailed assessment of multiple HDL-related biomarkers focusing on readily available parameters (HDL-C, apoA-I), and the confirmation of the prognostic value of HDL-C in a large external validation cohort comprising 985 patients.

In conclusion, we report for the first time that HDL-related biomarkers predict the development of complications in stable cirrhosis, as well as predicting short-term mortality in the setting of AD. The prognostic value of HDL-C and apoA-I as single biomarkers was similar to currently used composite scores. Both HDL-C and apoA-I provided incremental prognostic information when added to MELD. Further research is needed to validate HDL-related biomarkers as predictors of the development of ACLF in cirrhosis.

Abbreviations

Apo, apolipoprotein; ACLF, acute-on-chronic liver failure; AD, acute decompensation; CEC, cholesterol efflux capacity; CLIF-C,

Chronic Liver Failure Consortium; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; HVPG, hepatic venous pressure gradient; IL, interleukin; INR, international normalized ratio; LPS, lipopolysaccharide; MELD, model for end-stage liver disease; PLT, platelet count; SC, stable cirrhosis; TNF-α, tumor necrosis factor-α; WBC, white blood cell count.

Financial support
This study was supported by the Austrian Science Fund FWF (FWF W1241, DK-MOLIN, P22976-B18 to G.M. and P24362 to VS).

Conflicts of interest
Alexander Gerbes received personal fees from CSL Behring, GRIFOLS, and Falk. All other authors declare that they have no conflicts of interest related to the study.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
MT, FR, VS, AH[1], JC, RES, GM: study design, data evaluation, manuscript writing and final review; MT, AT, EK, HS, TS: lipid assay; CP, AA: data management, statistical analysis; FR, VS, PD, AH[3,5], LB, MM, RP, TR, AG, PC, CA, RM, RES: patient recruitment, data collection and final manuscript review.

Acknowledgements
The authors wish to thank Lusik Balayan, Sabine Paulitsch, Andreas Posch and Andrea Streit for excellent technical assistance.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2020.01.026.

References
Author names in bold designate shared co-first authorship

[1] Pimpin L, Cortez-Pinto H, Negro F, Corbould E, Lazarus JV, Webber L, et al. Burden of liver disease in Europe: epidemiology and analysis of risk factors to identify prevention policies. J Hepatol 2018;69:718–735.
[2] Scaglione S, Kliethermes S, Cao G, Shoham D, Durazo R, Luke A, et al. The epidemiology of cirrhosis in the United States: a population-based study. J Clin Gastroenterol 2015;49:690–696.
[3] D'Arienzo A, Manguso F, Scaglione G, Vicinanza G, Bennato R, Mazzacca G. Prognostic value of progressive decrease in serum cholesterol in predicting survival in Child-Pugh C viral cirrhosis. Scand J Gastroenterol 1998;33:1213–1218.
[4] Cicognani C, Malavolti M, Morselli-Labate AM, Zamboni L, Sama C, Barbara L. Serum lipid and lipoprotein patterns in patients with liver cirrhosis and chronic active hepatitis. Arch Intern Med 1997;157:792–796.
[5] Rubies-Prat J, Masdeu S, Nubiola AR, Chacon P, Holguera C, Masana L. High-density lipoprotein cholesterol and phospholipids, and apoprotein A in serum of patients with liver disease. Clin Chem 1982;28:525–527.
[6] Etogo-Asse FE, Vincent RP, Hughes SA, Auzinger G, Le Roux CW, Wendon J, et al. High density lipoprotein in patients with liver failure; relation to sepsis, adrenal function and outcome of illness. Liver Int 2012;32:128–136.
[7] Poynard T, Abella A, Pignon JP, Naveau S, Leluc R, Chaput JC. Apolipoprotein AI and alcoholic liver disease. Hepatology 1986;6:1391–1395.
[8] Habib A, Mihas AA, Abou-Assi SG, Williams LM, Gavis E, Pandak WM, et al. High-density lipoprotein cholesterol as an indicator of liver function and prognosis in noncholestatic cirrhotics. Clin Gastroenterol Hepatol 2005;3:286–291.
[9] Pirillo A, Catapano AL, Norata GD. HDL in infectious diseases and sepsis. Handb Exp Pharmacol 2015;224:483–508.
[10] Grunfeld C, Marshall M, Shigenaga JK, Moser AH, Tobias P, Feingold KR. Lipoproteins inhibit macrophage activation by lipoteichoic acid. J Lipid Res 1999;40:245–252.
[11] Murch O, Collin M, Hinds CJ, Thiemermann C. Lipoproteins in inflammation and sepsis. I. Basic science. Intensive Care Med 2007;33:13–24.
[12] Camont L, Lhomme M, Rached F, Le Goff W, Negre-Salvayre A, Salvayre R, et al. Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: relevance to cellular cholesterol efflux, antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic functionalities. Arterioscler Thromb Vasc Biol 2013;33:2715–2723.
[13] Fessler MB, Parks JS. Intracellular lipid flux and membrane microdomains as organizing principles in inflammatory cell signaling. J Immunol 2011;187:1529–1535.
[14] Christou L, Pappas G, Falagas ME. Bacterial infection-related morbidity and mortality in cirrhosis. Am J Gastroenterol 2007;102:1510–1517.
[15] Trieb M, Horvath A, Birner-Gruenberger R, Spindelboeck W, Stadlbauer V, Taschler U, et al. Liver disease alters high-density lipoprotein composition, metabolism and function. Biochim Biophys Acta 2016;1861:630–638.
[16] Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. Gastroenterology 2013;144:1426–1437.e1–9.
[17] Vodnala D, Rubenfire M, Brook RD. Secondary causes of dyslipidemia. Am J Cardiol 2012;110:823–825.
[18] Ito Y, Satoh N, Ishii T, Kumakura J, Hirano T. Development of a homogeneous assay for measurement of high-density lipoprotein-subclass cholesterol. Clin Chim Acta 2014;427:86–93.
[19] Holzer M, Schilcher G, Curcic S, Trieb M, Ljubojevic S, Stojakovic T, et al. Dialysis modalities and HDL composition and function. J Am Soc Nephrol 2015;26:2267–2276.
[20] Trieb M, Kornej J, Knuplez E, Hindricks G, Thiele H, Sommer P, et al. Atrial fibrillation is associated with alterations in HDL function, metabolism, and particle number. Basic Res Cardiol 2019;114:27.
[21] Untersteller K, Meissl S, Trieb M, Emrich IE, Zawada AM, Holzer M, et al. HDL functionality and cardiovascular outcome among nondialysis chronic kidney disease patients. J Lipid Res 2018;59:1256–1265.
[22] Jalan R, Pavesi M, Saliba F, Amoros A, Fernandez J, Holland-Fischer P, et al. The CLIF consortium acute decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-on-chronic liver failure. J Hepatol 2015;62:831–840.
[23] Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Gines P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. J Hepatol 2014;61:1038–1047.
[24] DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–845.
[25] Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. BMJ 2015;350:g7594.
[26] Arroyo V, Garcia-Martinez R, Salvatella X. Human serum albumin, systemic inflammation, and cirrhosis. J Hepatol 2014;61:396–407.
[27] **Claria J**, **Stauber RE**, Coenraad MJ, Moreau R, Jalan R, Pavesi M, et al. Systemic inflammation in decompensated cirrhosis: characterization and role in acute-on-chronic liver failure. Hepatology 2016;64:1249–1264.
[28] Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646–649.
[29] Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. Hepatology 2000;31:864–871.
[30] Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology 2010;139:1246–1256.e1–5.
[31] Tandon P, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. Semin Liver Dis 2008;28:26–42.
[32] Gustot T, Durand F, Lebrec D, Vincent JL, Moreau R. Severe sepsis in cirrhosis. Hepatology 2009;50:2022–2033.
[33] Foreman MG, Mannino DM, Moss M. Cirrhosis as a risk factor for sepsis and death: analysis of the national hospital discharge survey. Chest 2003;124:1016–1020.
[34] Arabi Y, Ahmed QA, Haddad S, Aljumah A, Al-Shimemeri A. Outcome predictors of cirrhosis patients admitted to the intensive care unit. Eur J Gastroenterol Hepatol 2004;16:333–339.

[35] Mackle IJ, Swann DG, Cook B. One year outcome of intensive care patients with decompensated alcoholic liver disease. Br J Anaesth 2006;97:496–498.
[36] Iqbal AJ, Barrett TJ, Taylor L, McNeill E, Manmadhan A, Recio C, et al. Acute exposure to apolipoprotein A1 inhibits macrophage chemotaxis in vitro and monocyte recruitment in vivo. Elife 2016;5. https://doi.org/10.7554/eLife.15190.
[37] Levine DM, Parker TS, Donnelly TM, Walsh A, Rubin AL. In vivo protection against endotoxin by plasma high density lipoprotein. Proc Natl Acad Sci U S A 1993;90:12040–12044.
[38] Hubsch AP, Casas AT, Doran JE. Protective effects of reconstituted high-density lipoprotein in rabbit gram-negative bacteremia models. J Lab Clin Med 1995;126:548–558.
[39] McDonald MC, Dhadly P, Cockerill GW, Cuzzocrea S, Mota-Filipe H, Hinds CJ, et al. Reconstituted high-density lipoprotein attenuates organ injury and adhesion molecule expression in a rodent model of endotoxic shock. Shock 2003;20:551–557.
[40] Tazi KA, Bieche I, Paradis V, Guichard C, Laurendeau I, Dargere D, et al. In vivo altered unfolded protein response and apoptosis in livers from lipopolysaccharide-challenged cirrhotic rats. J Hepatol 2007;46:1075–1088.
[41] Thabut D, Tazi KA, Bonnefont-Rousselot D, Aller M, Farges O, Guimont MC, et al. High-density lipoprotein administration attenuates liver proinflammatory response, restores liver endothelial nitric oxide synthase activity, and lowers portal pressure in cirrhotic rats. Hepatology 2007;46:1893–1906.
[42] Ramirez MJ, Ibanez A, Navasa M, Casals E, Morales-Ruiz M, Jimenez W, et al. High-density lipoproteins reduce the effect of endotoxin on cytokine production and systemic hemodynamics in cirrhotic rats with ascites. J Hepatol 2004;40:424–430.
[43] Galbois A, Thabut D, Tazi KA, Rudler M, Mohammadi MS, Bonnefont-Rousselot D, et al. Ex vivo effects of high-density lipoprotein exposure on the lipopolysaccharide-induced inflammatory response in patients with severe cirrhosis. Hepatology 2009;49:175–184.
[44] Michael Gibson C, Korjian S, Tricoci P, Daaboul Y, Yee M, Jain P, et al. Safety and tolerability of CSL112, a reconstituted, infusible, plasma-derived apolipoprotein A-I, after acute myocardial infarction: the AEGIS-I trial (ApoA-I event reducing in ischemic syndromes I). Circulation 2016;134:1918–1930.