



Original Article

1,5-Anhydroglucitol Predicts Mortality in Patients with HBV-Related Acute-on-chronic Liver Failure



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Abstract

Background and Aims: 1,5-Anhydroglucitol (1,5AG) activity has been reported in chronic liver disease. Hepatitis B virus (HBV)-related acute-on-chronic liver failure (HBV-ACLF) patients have a high mortality. We aimed to discover the relationship between serum 1,5AG and the prognosis of HBV-ACLF. **Methods:** Serum 1,5AG levels were determined in 333 patients with HBV-ACLF, 300 without diabetes were allocated to derivation (n=206) and validation cohorts (n=94), and 33 were recruited to evaluate 1,5AG in those with diabetes. Forty patients with chronic hepatitis B, 40 with liver cirrhosis, and 40 healthy people were controls in the validation cohort. **Results:** In the derivation and validation cohorts, serum 1,5AG levels were significantly lower in nonsurvivors than in survivors. The AUC of 1,5AG for 28-day mortality was 0.811. In patients with diabetes, serum 1,5AG levels were also significantly lower in nonsurvivors than in survivors. In multivariate Cox regression analysis, serum 1,5AG levels were independently associated with 28-day mortality. A novel predictive model (ACTIG) based on 1,5AG, age, TB, cholesterol, and INR was derived to predict mortality. In ACTIG, the AUC for 28-day mortality was 0.914, which was superior to some prognostic score models. ACTIG was also comparable to those prognostic score models in predicting 6-month mortality. In mice with D-galactosamine/lipopolysaccharide-induced liver failure, 1,5AG levels were significantly reduced in serum and significantly increased in urine and liver tissue. **Conclusions:** Serum 1,5AG levels are a promising predictor of short-term mortality in HBV-ACLF patients. The 1,5AG distribution changed in mice with D-

galactosamine/ lipopolysaccharide-induced liver failure.

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Introduction

Acute-on-chronic liver failure (ACLF) is associated with high short-term mortality,^{1,2} and hepatitis B virus (HBV) is a frequent etiology of ACLF in Asia.³ The high mortality of HBV-ACLF had made it valuable to find novel markers to predict the prognosis.^{4,5} 1,5-Anhydroglucitol (1,5AG) is obtained mainly from food, and nearly 99.9% is reabsorbed by glucose transporters in renal tubules to form a stable metabolic pool.⁶ Because glucose competitively inhibits reabsorption,⁷ 1,5AG is used as a biomarker to reflect glucose control and postprandial plasma glucose in diabetes.⁸ 1,5AG activity has been reported in various diseases, including acute coronary syndrome, diabetes and end-stage renal disease.^{9–11}

Yamagishi *et al.* found that 1,5AG levels were significantly reduced in cirrhotic patients compared with healthy people,¹² Koga *et al.* reported decreased serum 1,5AG levels in chronic liver disease and that low serum 1,5AG levels were linked to impaired liver function.¹³ 1,5AG can be biosynthesized in the liver,^{6,13,14} and previous research from our laboratory on plasma metabolites indicated that 1,5AG was a potential marker of liver regeneration in a rat model.¹⁵ However, a relationship between HBV-ACLF and serum 1,5AG levels has not been reported. Here, we explored the correlation between serum 1,5AG levels and the prognosis of HBV-ACLF for the first time. We confirmed our results in a mouse model of D-galactosamine- and lipopolysaccharide (D-GaIn/LPS)-induced liver failure.

Methods

Study population

A group of 384 HBV-ACLF patients were recruited from May

Keywords: Acute-on-chronic liver failure; 1,5-anhydroglucitol; Prognosis.

Abbreviations: 1, 5AG, 1,5-anhydroglucitol; ACLF, acute-on-chronic liver failure; AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the receiver operating curve; BUN, blood urea nitrogen; CHB, chronic hepatitis B; CLIF-C ACLF, Chronic Liver Failure Consortium Acute-on-Chronic Liver Failure score; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B; D-GaIn, D-galactosamine; GGT, γ -glutamyl transpeptidase; HBV, hepatitis B virus; HC, healthy controls; HE, hepatic encephalopathy; HR, hazard ratio; INR, international normalised ratio; LC, liver cirrhosis; LPS, lipopolysaccharide; MELD, Model for End-stage Liver Disease score; TB, total bilirubin; TBA, total bile acid.

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1, 2017, to December 31, 2020 at the First Affiliated Hospital, College of Medicine, Zhejiang University. Fifty-one were excluded, 300 without diabetes were separated into derivation ($n=206$) and validation cohorts ($n=94$), and a third cohort of 33 patients was recruited to evaluate 1,5AG in those with diabetes. Forty healthy controls (HC), 40 liver cirrhosis (LC) patients, and 40 chronic hepatitis B (CHB) patients were controls in the validation cohort. Exclusion characteristics were the presence of renal disease, hepatocellular carcinoma, extrahepatic malignancies, <18 years of age, pregnancy, other serious systemic diseases pre-existing liver disease (autoimmune liver disease, schistosomiasis disease, alcoholic liver disease, and so on). Fifty-one patients were excluded. Sixteen did not satisfy the Asian Pacific Association for the Study of the Liver ACLF criteria, five for lack of follow-up, six for hepatocellular carcinoma, three for extrahepatic malignancies, fourteen for pre-existing liver diseases, two for pre-existing renal diseases, and five for incomplete data.

HBV-ACLF was diagnosed by the Asian Pacific Association for the Study of the Liver criteria as previously described.¹⁶ CHB was diagnosed by the 2009 American Association for the Study of Liver Diseases guidelines, and LC was diagnosed according to the 2012 North American consortium for the study of end-stage liver disease experience.¹⁷⁻¹⁹ Prognostic score models, including the Model for End-Stage Liver Disease (MELD),²⁰ iMELD,²¹ MELD-Na,²² Chinese Group on the Study of Severe Hepatitis B (COSSH-ACLF),²³ and Chronic Liver Failure Consortium Acute-on-Chronic Liver Failure (CLIF-C ACLF)²⁴ were used to predict 28-day and 6-month mortality.

The study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University and was conducted in compliance with the principles of the 1975 Declaration of Helsinki. Subjects gave informed consent to participate in the study. All animal experiments were performed under sodium pentobarbital anesthesia to minimize suffering and were approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine.

Animals

Male C57BL/6J mice (20–25 g) were obtained from Beijing Vital River Laboratory Animal Technology Corporation. Liver injury was induced by injection of 400 mg/kg D-GaIN and 10 µg/kg LPS (Sigma, St Louis, MO, USA). Mice were sacrificed 6 h after injection, and liver tissue, urine and blood were collected for analysis. The survival of mice in the study groups was continuously monitored every 2 h for 24 h, and 24 mice (12 per group) were used.

Biochemical assays

Serum, liver tissue, and urine were stored at -80°C before use. 1,5AG was determined with ELISA kits (Abbexa, Cambridge, UK). ALT, AST, and serum glucose levels were determined with commercial kits (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Statistical analysis

Statistical analysis was performed with MEDCALC (Ostend, Belgium) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were reported as means \pm standard deviation or medians with interquartile range. Differences were compared with Student's *t*-test, Mann-Whitney

U tests, one-way analysis of variance or Kruskal-Wallis H tests, as appropriate. Categorical variables were reported as numbers and percentages (%), and differences were compared with chi-squared or Fisher's exact tests. Multivariate analysis of independent prognostic factors for HBV-ACLF was performed using Cox regression. Cumulative survival rates were compared by the Kaplan-Meier method and log-rank tests.

Results

Patient baseline characteristics

From 1 May 2017, to 31 December 2020, 384 patients with HBV-ACLF were enrolled. Based on the exclusion criteria, 51 were not included in the analysis, 83.5% of the derivation cohort. were men, and 34.0% died or underwent liver transplantation within 28 days. At 28 days the nonsurvivors were older ($p=0.005$), had higher white blood cell ($p=0.008$) and neutrophil ($p=0.002$) counts, higher total bilirubin (TB, $p<0.001$), hepatic encephalopathy (HE) grade ($p<0.001$), hypoglycemia ($p=0.040$), international normalized ratio (INR, $p<0.001$), ammonia ($p=0.002$), and blood urea nitrogen (BUN, $p=0.005$) than the survivors (Table 1). 1,5AG ($p<0.001$), triglycerides ($p<0.001$), cholesterol ($p<0.001$), alpha fetoprotein (AFP, $p=0.001$), and gamma glutamyl transpeptidase (GGT, $p=0.007$) were significantly higher in survivors than in nonsurvivors (Table 1). The incidence of organ failure including the liver ($p=0.004$), brain ($p=0.006$), and coagulation ($p<0.001$), was significantly higher in nonsurvivors (Table 1). The MELD, iMELD, MELD-Na, COSSH-ACLF and CLIF-C ACLF scores were also significantly higher in nonsurvivors (Table 1, all $p<0.001$). The clinical data of the validation cohort are shown in Supplementary Table 1.

Serum 1,5AG levels in HBV-ACLF

As shown in Figure 1A, serum 1,5AG levels were lower in nonsurvivors than in survivors ($p<0.001$) in the derivation and validation cohorts. As the number of failed organs increased in the derivation cohorts, the serum 1,5AG levels significantly decreased ($p<0.001$, Fig. 1B). In the validation cohort, serum 1,5AG was significantly lower in HBV-ACLF nonsurvivors than in survivors and in the HC, CHB, and LC groups ($p<0.05$, Fig. 1C). 1,5AG levels were higher in the HC than in the CHB and LC groups and the HBV-ACLF survivors and nonsurvivors ($p<0.05$, Fig. 1C). The characteristics of patients in the HC, CHB, and LC groups are shown in Supplementary Table 2. Patients in the LC group were older than those in the CHB, HC and HBV-ACLF groups. There were no significant sex differences of the four groups.

1,5AG levels in HBV-ACLF in 33 patients with diabetes are shown in Figure 1I. Serum 1,5AG levels were significantly lower in nonsurvivors ($p<0.05$) and lower in those with diabetes than without diabetes ($p<0.01$, Fig. 1H). In HBV-ACLF survivors, serum 1,5AG levels were also significantly lower in patients with diabetes ($p<0.01$, Supplementary Fig. 1A). In the nonsurvivor groups, there were no significant differences in serum 1,5AG levels between HBV-ACLF patients with and without diabetes ($p=0.457$, Supplementary Fig. 1B). The clinical characteristics of patients with diabetes are shown in Supplementary Table 3.

Correlations of serum 1,5AG levels and the laboratory values in the derivation cohort are shown in Supplementary Figure 3. Serum 1,5AG had a moderately positive correlation with serum uric acid ($r=0.307$) in patients without pre-existing hyperuricemia and a moderately negative

Table 1. Baseline characteristics of the derivation cohort

Characteristic	HBV-ACLF (n=206)		p
	Survivors (n=136)	Nonsurvivors (n=70)	
Age, years	45.4±12.6	51.2±10.5	0.005
Male sex	109 (80.1)	63 (90.0)	0.071
BMI (kg/m ²)	24.0±3.6	23.5±3.6	0.358
Liver cirrhosis	72 (52.9)	41 (58.6)	0.442
Complications			
Gastrointestinal hemorrhage	5 (3.7)	7 (10.0)	0.128
Ascites	74 (54.4)	38 (54.3)	0.986
Infection	55 (40.4)	33 (47.1)	0.357
HE			
I–II	14 (10.3)	19 (27.1)	<0.001
III–IV	4 (2.9)	10 (14.3)	
Laboratory data			
ALT (U/L)	160.5 (89.0, 376.5)	255.0 (105.5, 405.0)	0.258
AST (U/L)	128.5 (76.0, 214.0)	147.0 (88.5, 230.8)	0.263
ALP (U/L)	133.0 (108.0, 157.5)	126.0 (107.8, 150.8)	0.455
Albumin (g/dL)	31.2±4.0	31.7±4.5	0.478
TB (µmol/L)	327.9±109.8	412.4±126.5	<0.001
TBA (µmol/L)	280.0±116.8	264.5±116.1	0.371
GGT (U/L)	79.0 (57.0, 116.5)	62.0 (41.8, 106.0)	0.007
Creatinine (µmol/L)	66.0 (55.0, 75.0)	65.5 (55.0, 94.5)	0.143
BUN (mmol/L)	4.0 (2.9, 5.4)	4.7 (3.4, 6.8)	0.005
Triglycerides (mmol/L)	1.3 (1.1, 1.8)	1 (0.8, 1.3)	<0.001
Uric acid (µmol/L)	143.1±45.9	130.0±47.6	0.057
Cholesterol (mmol/L)	2.4±0.9	2.0±0.7	<0.001
White blood cell count (10 ⁹ /L)	7.1±3.0	8.3±3.6	0.008
Neutrophil count (10 ⁹ /L)	4.9±2.7	6.3±3.3	0.002
Hemoglobin (g/L)	122.3±23.0	123.0±19.7	0.827
Platelet count (10 ⁹ /L)	112.5±57.4	101.6±51.2	0.183
INR	1.8 (1.6, 2.1)	2.6 (2.1, 3.1)	<0.001
Lg (DNA)	5.2±1.6	5.9±1.9	0.010
Ferritin (ng/mL)	2,587.2 (1,525.5, 4,025.1)	3,149.8 (1,866.8, 5,184.5)	0.069
AFP (ng/mL)	154.5 (50.3, 353.8)	49.1 (18.7, 186.7)	0.001
Sodium (mmol/L)	137.7±3.4	137.8±4.6	0.852
Serum ammonia (µmol/L)	49.5±25.5	66.3±37.0	0.002
Blood glucose (mmol/L)	4.0 (3.4, 4.6)	4.3 (3.4, 5.6)	0.240
Hypoglycemia (no.)	8 (5.9)	10 (14.5)	0.040
1,5AG (µg /mL)	36.7±12.5	23.3±8.7	<0.001
Organ failure			
Liver	115 (84.6)	69 (98.6)	0.004
Coagulation	10 (7.4)	41 (58.6)	<0.001
Kidney	0 (0.0)	3 (4.3)	0.069
Brain	4 (2.9)	10 (14.3)	0.006

(continued)

Table 1. - (continued)

Characteristic	HBV-ACLF (n=206)		p
	Survivors (n=136)	Nonsurvivors (n=70)	
Lung	0 (0.0)	1 (1.4)	0.340
Circulation	0 (0.0)	1 (1.4)	0.340
Severity score			
MELD	21.2±3.7	27.4±6.4	<0.001
MELD-Na	21.9±4.4	28.5±7.0	<0.001
iMELD	38.4±6.3	46.4±8.6	<0.001
CLIF-C ACLF	37.6±6.4	46.6±7.4	<0.001
COSSH-ACLF	4.8±0.5	6.0±1.1	<0.001
HBV-ACLF (COSSH criteria)			
ACLF grade 1	126 (92.6)	28 (40.0)	<0.001
ACLF grade 2	9 (6.6)	30 (42.9)	
ACLF grade 3	1 (0.7)	12 (17.1)	

Data are means ± standard deviation, numbers (%), or medians (interquartile range), as shown. 1,5AG, 1,5-anhydroglucitol; AFP, Alpha fetoprotein; ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BUN, Blood urea nitrogen; CLIF-C ACLF, Chronic Liver Failure Consortium Acute-on-Chronic Liver Failure score; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B; GGT, γ -glutamyl transpeptidase; HE, Hepatic encephalopathy; iMELD, Integrated MELD. INR, International normalized ratio; MELD-Na, MELD sodium; MELD, Model for End-stage Liver Disease score; TB, Total bilirubin; TBA, Total bile acid.

correlation with INR ($r = -0.353$, Supplementary Fig. 3A). Serum 1,5AG levels were not significantly correlated with AFP ($r = 0.078$), BMI ($r = 0.095$), blood glucose ($r = -0.017$), BUN ($r = -0.122$), TB ($r = -0.106$), or creatinine ($r = -0.124$, Supplementary Fig. 3A). Serum 1,5AG levels were also negatively correlated with COSSH-ACLF ($r = -0.370$), CLIF-C ACLF ($r = -0.327$), MELD ($r = -0.323$), iMELD ($r = -0.318$), and MELD-Na ($r = -0.312$) prognosis scores (Supplementary Fig. 3B). We confirmed the correlations in the validation cohort (Supplementary Fig. 4).

Predictive ability of serum 1,5AG levels for 28-day and 6-month mortality

The predictive ability of serum 1,5AG levels for 28-day mortality was investigated in the derivation cohorts. The area under the receiver operating characteristic curve (AUC) of serum 1,5AG levels alone was 0.811 (Fig. 1D). Patients were stratified into low- and high-1,5AG groups using the optimal 1,5AG cutoff value of 29.5 $\mu\text{g/mL}$. The 28-day mortality was significantly higher in the low-1,5AG group ($p < 0.001$, Fig. 1F). The sensitivity was 0.800 and specificity was 0.654. We confirmed the results in the validation cohort (Supplementary Fig. 2A and C). The predictive ability of serum 1,5AG in HBV-ACLF patients with diabetes was 0.763 (Supplementary Fig. 1C).

Six-month mortality was estimated in the derivation, validation, and HBV-ACLF with diabetes cohorts. Four patients lost to follow-up. The predictive ability of serum 1,5AG levels alone was 0.737 in the derivation cohort (Fig. 1E), 0.728 in the validation cohort (Supplementary Fig. 2B) and 0.688 in the HBV-ACLF with diabetes cohort (Supplementary Fig. 1D). Mortality was significantly higher in the low-1,5AG than in the high-1,5AG group in the derivation and validation cohorts ($p < 0.001$; Figs 1G and 2D).

Independent prognostic predictors for short-term mortality

In the derivation cohort, univariate Cox analysis found that

HE grades, age, TB, creatinine, triglycerides, cholesterol, white blood cell count, neutrophil count, INR, Lg (DNA), ferritin, AFP, and serum 1,5AG levels (Table 2) were high-risk factors for the 28-day mortality of HBV-ACLF. Multivariate Cox regression indicated that serum 1,5AG level [(hazard ratio (HR) 0.918, $p < 0.001$), TB level (HR 1.003, $p = 0.003$), age (HR 1.026, $p = 0.022$), INR (HR 3.724, $p < 0.001$) and cholesterol level (HR 0.619, $p = 0.007$)] were independent prognostic predictors of 28-day mortality of HBV-ACLF (Table 2).

A predictive model was developed from the multivariate Cox regression analysis with $\text{ACTIG} = 0.026 \times \text{age} + 0.003 \times \text{TB} + 1.315 \times \text{INR} - 0.085 \times 1,5\text{AG} - 0.480 \times \text{cholesterol}$. The AUC for 28-day mortality of the ACTIG model was 0.914, which was superior to those of MELD-Na (0.801, $p < 0.001$), iMELD (0.774, $p < 0.001$), MELD (0.812, $p < 0.001$), COSSH-ACLF (0.861, $p = 0.028$) and CLIF-C ACLF (0.825, $p = 0.002$) for predicting 28-day mortality (Table 3, Fig. 2A). In predicting 6-month mortality (Table 3, Fig. 2B), ACTIG (0.865) was also comparable to that of MELD-Na (0.818, $p = 0.147$), iMELD (0.805, $p = 0.069$), MELD (0.825, $p = 0.206$), COSSH-ACLF (0.857, $p = 0.751$) and CLIF-C ACLF (0.820, $p = 0.136$). Nonsurvivors had a significantly higher ACTIG score than survivors in the derivation and validation cohorts (Fig. 2C). We divided patients into high- and low-ACTIG groups by the cutoff value of 0.710. The 28-day and 6-month mortality of the high-ACTIG group was significantly higher than that of the low-ACTIG group ($p < 0.001$, Fig. 2D and E). We confirmed the new model in the validation cohort (Supplementary Table 4, Fig. 2G and H) and HBV-ACLF patients with diabetes (Supplementary Fig. 1E and F).

1,5AG levels in D-GaIN/LPS-induced liver failure

Previous studies reported that 1,5AG remains steady in blood, tissues and urine.^{25,26} Therefore, the distribution balance of 1,5AG may be disrupted in ACLF patients. As HBV-ACLF does not have a standard animal model, we investigated 1,5AG levels in D-GaIN/LPS-induced liver failure. LPS/D-GaIN-treated mice began to die at 6 h, and the mortality rate was 75% (Fig. 3B). As shown in Figure 3B, serum ALT ($p = 0.014$) and AST ($p < 0.001$) levels were significantly increased in the D-

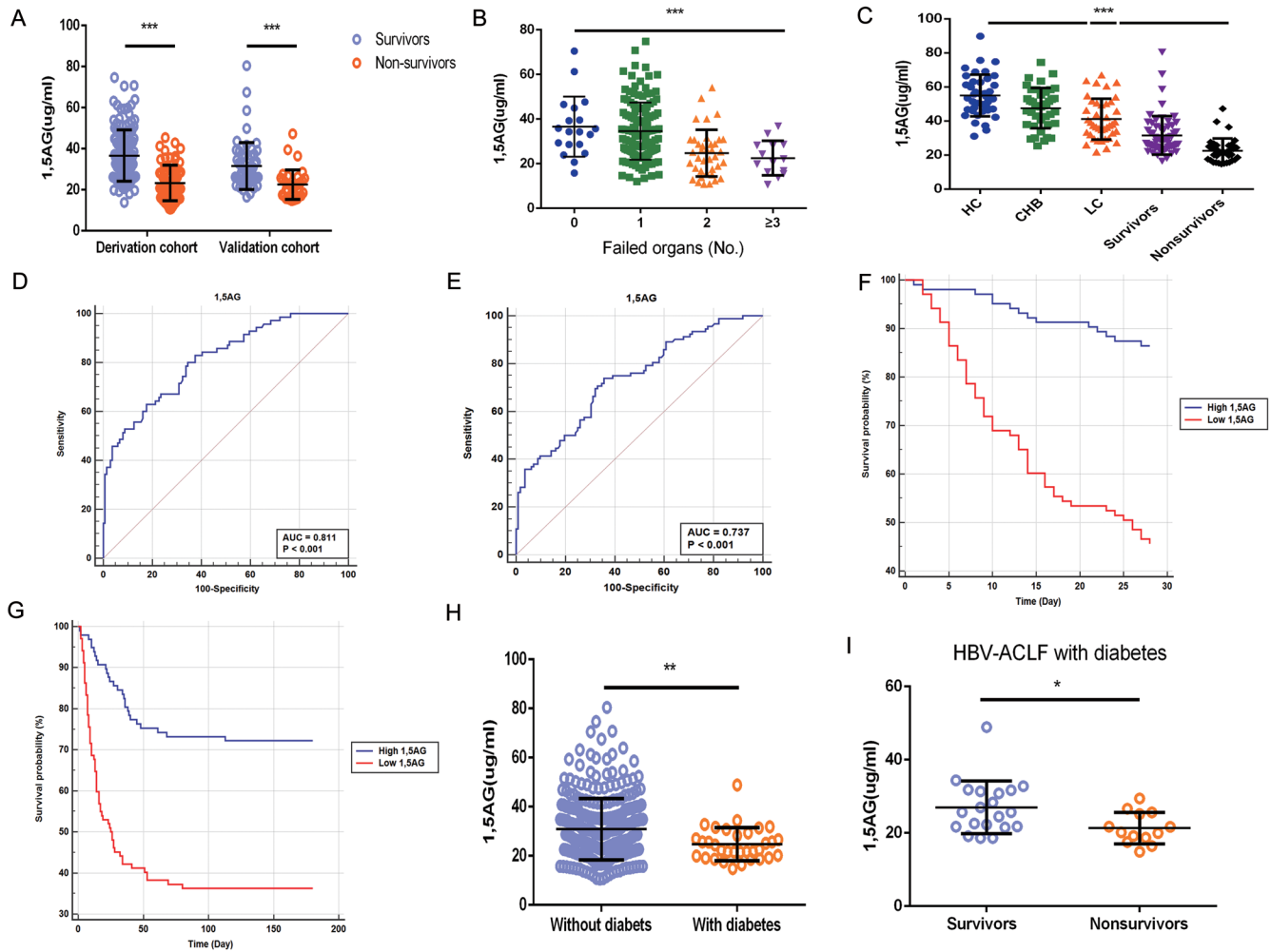


Fig. 1. Serum 1,5-Anhydroglucitol (1,5AG) levels in patients with hepatitis B virus related acute-on-chronic liver failure (HBV-ACLF) and ability to predict 28 day and 6-month mortality. Distribution of serum 1,5AG levels in the derivation and validation cohorts (a). Serum 1,5AG levels decreased with increasing failed organ numbers in the derivation cohort (b). Serum 1,5AG levels were significantly lower in HBV-ACLF nonsurvivors than in the survivors and the healthy controls (HC), chronic hepatitis B (CHB), and liver cirrhosis (LC) groups (c). Prediction of 28-day (d) and 6-month (e) mortality by serum 1,5AG levels in the derivation cohorts. Survival rates at 28 days (f) and 6 months (g) at high- and low serum 1,5AG levels in the derivation cohort. Serum 1,5AG levels in HBV-ACLF patients with and without diabetes (h). Serum 1,5AG level distribution in HBV-ACLF patients with diabetes (i). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

GaIN/LPS group at 6 h. Hematoxylin–eosin staining showed liver architecture disruption, vacuolization, disappearance of nuclei, ballooned hepatocytes, and severe tissue hemorrhage (Fig. 3A). Assays of 1,5AG concentration in serum, urine and liver tissue found that the 1,5AG levels were significantly reduced in serum ($p = 0.001$, Fig. 3C) and significantly increased in urine ($p = 0.005$, Fig. 3C) and liver tissue ($p = 0.009$, Fig. 3C) in the D-GaIN/LPS group. We also found lower glucose levels in the D-GaIN/LPS group ($p < 0.001$, Fig. 3B).

Discussion

Because of the high mortality of liver failure,¹ accurate biomarkers of prognosis are valuable. Here, we showed for the first time that serum 1,5AG levels were predictive of the 28-day and 6-month mortality of HBV-ACLF patients. We showed that serum 1,5AG levels were also significantly lower in HBV-ACLF nonsurvivors than in the survivors and the HC, CHB, and LC groups. In accord with previous studies,^{12,13} we found lower serum 1,5AG levels in the LC and CHB groups than

in the HC group. According to the optimal cutoff value, patients with lower serum 1,5AG levels had significantly higher 28 day and 6-month mortality. The results were validated in another cohort of HBV-ACLF patients, which strengthens the reliability of the predictive ability. It is well known that 1,5AG is related to diabetes. Thus, we also investigated 1,5AG levels in HBV-ACLF patients with diabetes. Serum 1,5AG levels were significantly lower in HBV-ACLF patients with diabetes. In patients with diabetes, we also found that serum 1,5AG levels were markedly lower in nonsurvivors than in survivors. Because of the small sample size, the association between serum 1,5AG levels and the prognosis of HBV-ACLF with diabetes needs to be confirmed in further studies.

Moreover, in multivariate Cox regression analysis of 28-day mortality, serum 1,5AG levels remained an independent prognostic factor when analyzed together with clinical parameters. We developed a new predictive model that was superior to MELD-Na, iMELD, MELD, COSSH-ACLF and CLIF-C ACLF in predicting 28-day mortality. It was also comparable to MELD-Na, iMELD, MELD, COSSH-ACLF and CLIF-C ACLF in predicting 6-month mortality. To our knowledge, there are only a few

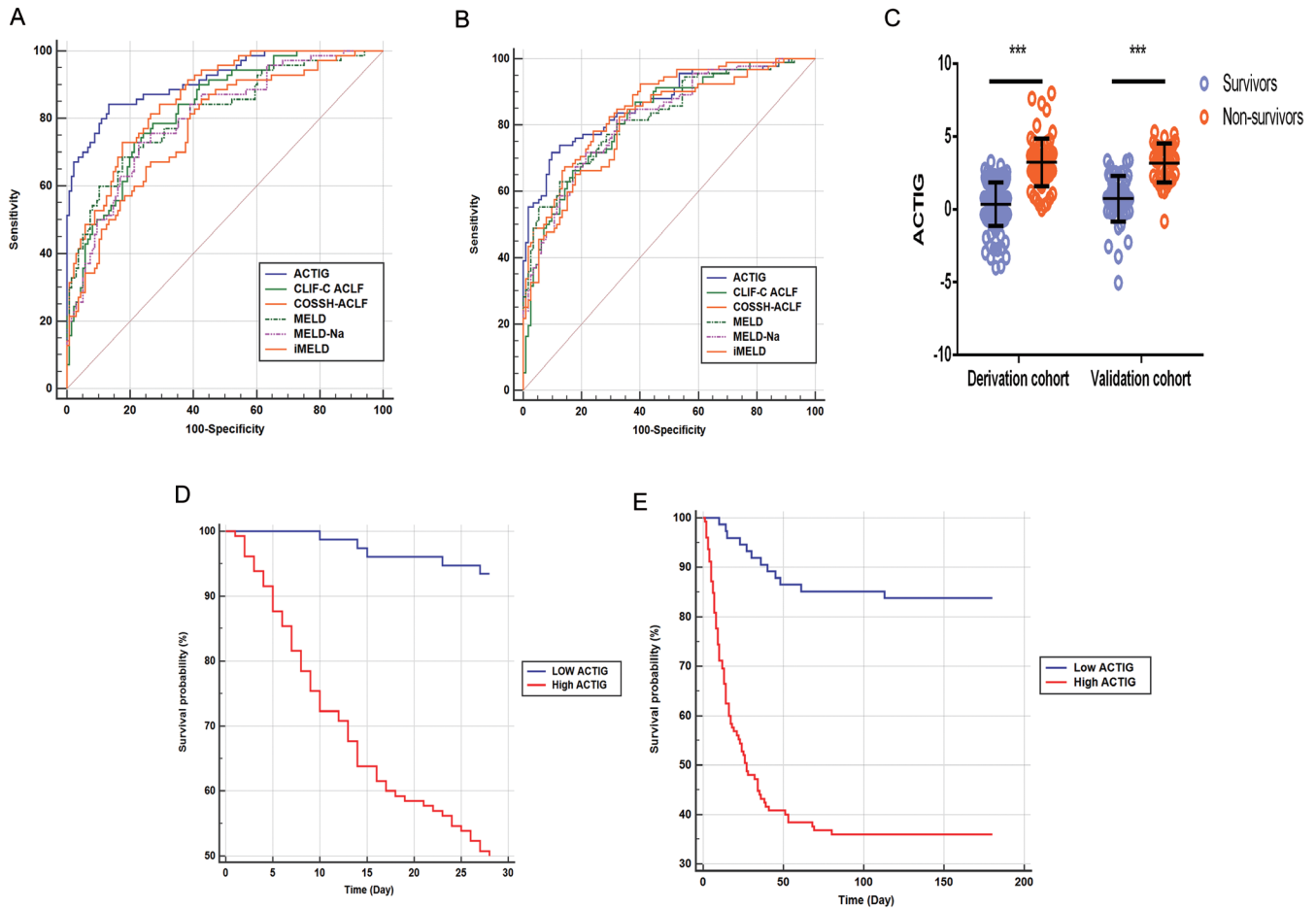


Fig. 2. Prediction of 28 day and 6-month mortality of patients with HBV-ACLF, by ACTIG and other scores. Prediction of 28 day (a), and 6-month (b), mortality in the derivation cohorts. ACTIG score distribution in both the derivation and validation cohorts (c). Survival rates at 28 days (d), and 6 months (e), in the low- and high-ACTIG score groups in the derivation cohort. *** $p < 0.001$.

Table 2. Independent risk factors for 28-day mortality

Variable	Univariate Cox regression model		Multivariate Cox regression model	
	HR (CI 95 %)	<i>p</i>	HR (CI 95 %)	<i>p</i>
Age, years	1.030 (1.011–1.050)	0.002	1.026 (1.004–1.049)	0.022
HE	2.248 (1.647–3.068)	<0.001		
TB (μmol/L)	1.005 (1.003–1.007)	<0.001	1.003 (1.001–1.005)	0.003
Creatinine (μmol/L)	1.012 (1.007–1.016)	<0.001		
Triglycerides (mmol/L)	0.233 (0.117–0.465)	<0.001		
Cholesterol (mmol/L)	0.526 (0.364–0.761)	0.001	0.619 (0.437–0.876)	0.007
White blood cell count (10 ⁹ /L)	1.101 (1.032–1.175)	0.003		
Neutrophil count (10 ⁹ /L)	1.129 (1.056–1.207)	<0.001		
INR	2.901 (2.352–3.577)	<0.001	3.724 (2.583–5.368)	<0.001
Lg (DNA)	1.203 (1.048–1.382)	0.009		
Ferritin	1.000 (1.000–1.000)	0.020		
AFP	0.999 (0.997–1.000)	0.016		
1,5AG	0.904 (0.878–0.930)	<0.001	0.918 (0.890–0.948)	<0.001

1,5AG, 1,5-anhydroglucitol; AFP, Alpha fetoprotein; HE, Hepatic encephalopathy; INR, International normalized ratio; TB, Total bilirubin; HR, Hazard ratio.

Table 3. Comparison of the predictive value for patients with HBV-ACLF in derivation cohorts

Model	28-day mortality			6-month mortality		
	AUC (95% CI)	Z-value	p-value	AUC (95% CI)	Z-value	p-value
<i>n</i> =206				<i>n</i> =204		
ACTIG	0.914 (0.867–0.948)			0.865 (0.810–0.908)		
MELD	0.812 (0.752–0.863)	3.464	<0.001	0.825 (0.766–0.875)	1.266	0.206
MELD-Na	0.801 (0.740–0.853)	3.753	<0.001	0.818 (0.759–0.869)	1.452	0.147
iMELD	0.774 (0.710–0.829)	4.497	<0.001	0.805 (0.744–0.857)	1.816	0.069
CLIF-C ACLFs	0.825 (0.766–0.874)	3.085	0.002	0.820 (0.760–0.870)	1.490	0.136
COSSH-ACLFs	0.861 (0.806–0.905)	2.204	0.028	0.857 (0.801–0.902)	0.318	0.751

CLIF-C ACLF, Chronic Liver Failure Consortium Acute-on-Chronic Liver Failure score; COSSH-ACLF: Chinese Group on the Study of Severe Hepatitis B; MELD: Model for End-stage Liver Disease score; MELD-Na: MELD sodium; iMELD: Integrated MELD.

studies evaluating the association of 1,5AG level with liver diseases.^{12,13} 1,5AG levels have not been studied thus far in ACLF patients. All the results demonstrated that the serum 1,5AG level is a promising biomarker for HBV-ACLF.

As an animal model, LPS/GaIN has been widely used to mimic clinical liver dysfunction.²⁷ We found that 1,5AG levels were significantly reduced in serum and significantly increased in urine and liver tissue in mice with D-GaIN/LPS-induced liver failure. That indicates that the distribution balance of 1,5AG was changed. Small amounts of 1,5AG are synthesized in the liver by the anhydrofructose pathway.¹⁴

However, most 1,5AG originates from food, so impaired hepatic synthesis cannot explain the reduced serum 1,5AG levels.¹³ It also cannot explain the increase in 1,5AG levels in the liver in our mouse model. It has been reported that 1,5AG remains balanced in blood, tissues and urine.²⁵ Koga *et al.*¹³ found a correlation between 1,5AG levels and serum uric acid.²⁸ Uric acid is also reabsorbed in renal tubules. Therefore, they proposed that the low serum 1,5AG levels in patients with chronic liver disease may be associated with renal tubular dysfunction. According to a previously published study, patients with jaundice developed hypouricemia that

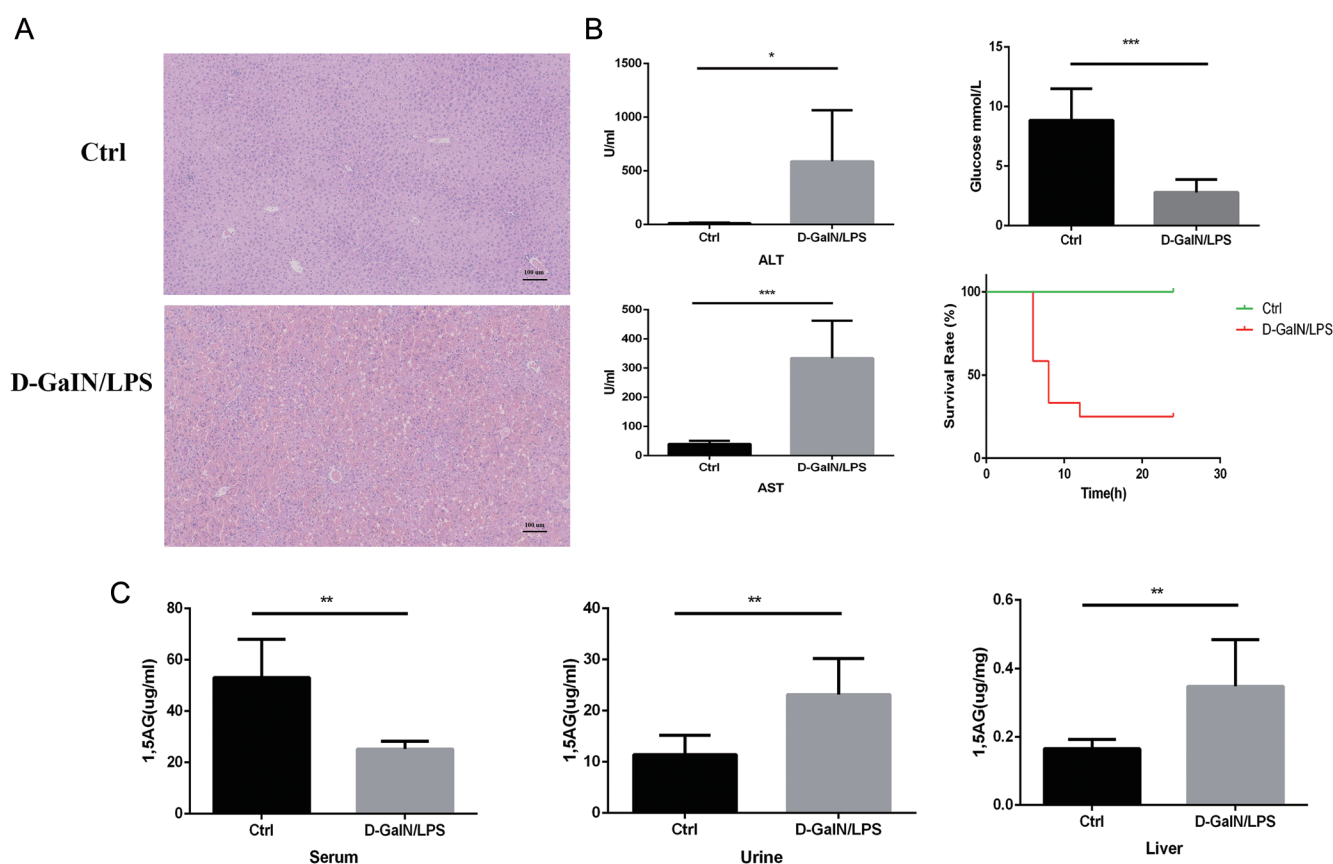


Fig. 3. 1,5AG concentration in the D-galactosamine and lipopolysaccharide (D-GaIN/LPS)-induced liver failure model. Hematoxylin–eosin-stained liver sections (100×). (a) Serum glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in mice (*n*=6). (b) Survival rate of D-GaIN/LPS-induced liver failure (*n*=12). (c) 1,5AG concentration in serum (*n*=6), urine (*n*=6) and liver tissue (*n*=6). (c) **p*<0.05, ***p*<0.01 and ****p*<0.001.

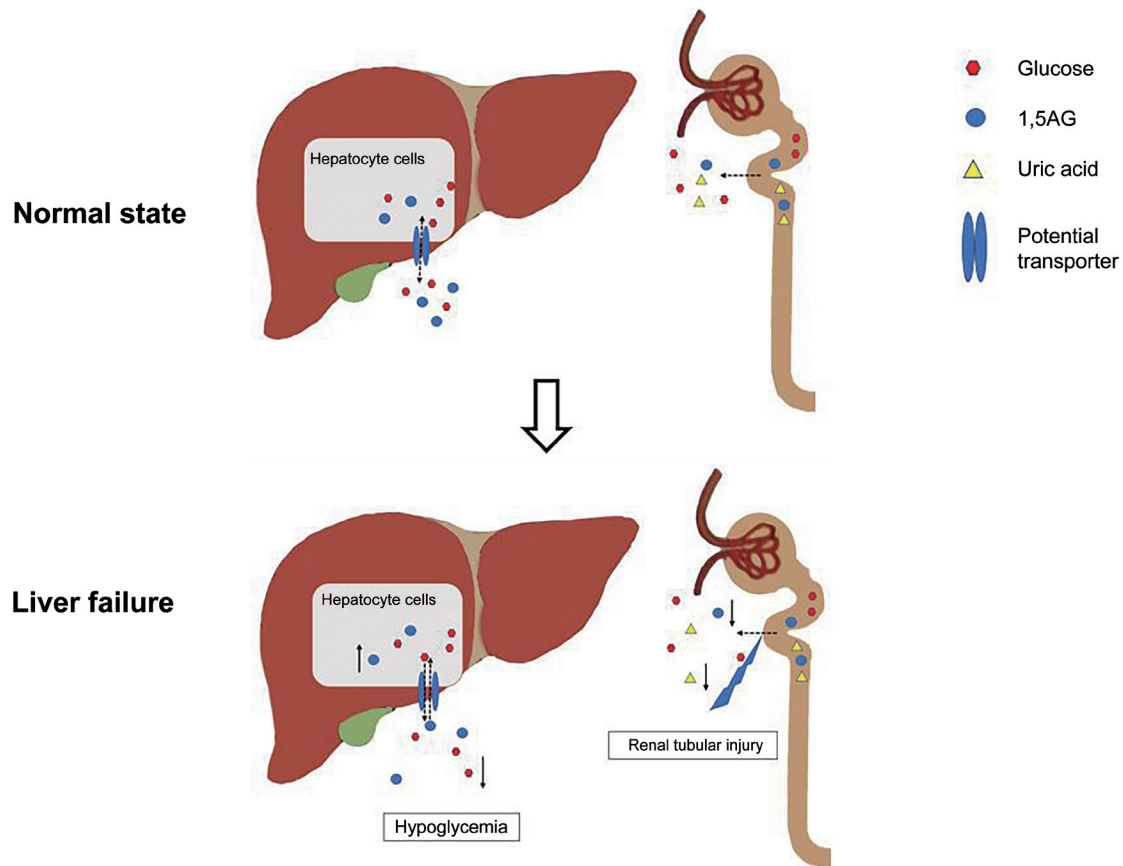


Fig. 4. Potential low-level expression of 1,5-Anhydroglucitol (1,5AG) in liver failure. 1,5AG, glucose and uric acid are reabsorbed in renal tubules. 1,5AG and glucose may share the same transporter and are competitive with each other in hepatocytes. Renal tubular injury decreases the reabsorption of 1,5AG and uric acid. The reduced blood glucose level causes 1,5AG to flow into hepatocytes.

was associated with renal tubular dysfunction.²⁹ However, they did not investigate urinary excretion of 1,5AG in chronic liver disease patients. Another study also found that serum 1,5AG and urinary excretion 1,5AG were closely correlated with urine N-acetyl-beta-glucosaminidase, which may indicate renal tubular damage.³⁰ Renal injury is a common complication of ACLF and is always related to poor outcomes.³¹ We found a correlation between uric acid and serum 1,5AG levels in our HBV-ACLF patients. In our mouse model, 1,5AG levels were significantly increased in urine, but we did not find significant correlations between 1,5AG and creatinine and BUN. Creatinine is a marker of glomerular filtration function but not kidney tubular injury.³² BUN cannot accurately reflect kidney function in patients with end-stage liver disease.³³ Based on those studies and our results, low serum 1,5AG levels in HBV-ACLF patients may be partly related to the decrease in reabsorption of 1,5AG caused by renal tubular injury, as illustrated by Figure 4. A future study may be required to detect specific biomarkers of renal tubular injury may be required to confirm the results.

However, the significantly increased 1,5AG levels in liver tissue still need to be explained. Serum 1,5AG levels were reported to dramatically increase during the oral glucose tolerance test.³⁴ An *in vitro* study found that after an acute glucose load, 1,5AG in the culture medium of hepatocytes increased slightly rather than declining. 1,5AG and glucose may share the same transporter and are competitive with each other in hepatocytes.²⁶ It is known that glucose metabolism is impaired during liver disease.³⁵ Our mouse mod-

el showed hypoglycemia, which may explain the increased 1,5AG levels in liver tissue. The HBV-ACLF nonsurvivor group also displayed a higher percentage of hypoglycemia. However, we did not find a significant relation between serum 1,5AG levels and blood glucose. The relationship between serum 1,5AG levels and blood glucose in ACLF should be investigated in the future.

Several study limitations may have influenced the results. First, the prognostic predictive ability of serum 1,5AG levels was only observed in a single center study with a retrospective cohort study. The result should be assessed in multicenter prospective studies. Second, our research only included ACLF patients with HBV infection, and the prognostic value of 1,5AG in liver failure caused by other etiologies needs further confirmation. Third, the D-GaIN/LPS-induced liver failure model cannot fully reflect the pathophysiological changes in ACLF patients, and we may need a better model to verify the results.

In summary, serum 1,5AG levels were a promising predictor of short-term mortality in patients with HBV-ACLF. 1,5AG distribution changed in a D-GaIN/LPS-induced liver failure model.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (LL), acquisition of data and performing the experiments (LZ, YZ, ZX), analysis and interpretation of data (LZ, LX, QH), drafting the manuscript (LZ, QL, ST), review and editing (JW). All authors read and approved the final manuscript.

Data sharing statement

No additional data are available.

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