Genotype Matters in Patients with Acute-on-chronic Liver Failure Due to Reactivation of Chronic Hepatitis B

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Abstract

Background: Acute-on-chronic liver failure (ACLF) can be caused by reactivation of chronic hepatitis B virus (HBV) infection (HBV-ACLF). It’s unclear whether HBV genotypes affect the clinical and therapeutical outcomes of patients with HBV-ACLF. This study was to investigate the short-term antiviral response and overall survival in HBV-ACLF patients treated by tenofovir or entecavir.

Methods: Seventy-three consecutive patients with HBV-ACLF were stratified into genotype B group (n = 33) and C group (n = 40). They were prospectively followed-up.

Results: At 2 weeks, the genotype B group had significantly lower HBV-DNA load (P = 0.005), greater HBV-DNA decline (P = 0.026), higher proportion of patients with HBV-DNA < 500 IU/ml (P = 0.007), improved Child-Turcotte-Pugh (CTP; P = 0.032) and model for end-stage liver disease (MELD; P = 0.039) scores compared to the genotype C group. At three months, survivors in both groups had undetectable HBV-DNA loads, comparable CTP (P = 0.850) and MELD (P = 0.861) scores; the genotype C group had markedly lower overall survival rate than the B group (P = 0.013). The genotype (hazard ratio [HR]: 2.138; 95% confidence interval [CI]: 1.034–4.143; P = 0.041), MELD score (HR: 1.664, 95% CI: 1.077–2.571; P = 0.022) and HBV-DNA decline (HR: 0.225, 95% CI: 0.067–0.758; P = 0.016) at 2 weeks were significantly associated with mortality at 3 months. No severe adverse event was noted.

Conclusions: Genotype B was associated with better short-term antiviral response and clinical outcome compared to genotype C in patients with HBV-ACLF.

Introduction

Approximately 360 million people worldwide were chronically infected by hepatitis B virus (HBV) that causes a wide spectrum of diseases, including inactive carrier, persistent chronic hepatitis B (CHB), cirrhosis, hepatocellular carcinoma (HCC) and liver failure1,2. On the basis of chronic liver disease, some patients may have acute and severe deterioration of liver function, progressing to liver failure, namely acute-on-chronic liver failure (ACLF), which can be caused by a number of precipitating events, such as bacterial infection, HBV reactivation, and hepatitis viruses superimposed infection, active alcoholism, surgery, and hepatotoxic drugs3. ACLF caused by reactivation of chronic HBV infection (HBV-ACLF) is associated with high morbidity and mortality4. HBV reactivation is often spontaneous, but sometimes can be triggered by such factors as malignancy chemotherapy, immunosuppression, or antiviral resistance mutation.
HBV can be classified into eight unique genotypes (A–H) according to a more than 8% divergence of the entire genomic nucleotide sequence\(^5\)–\(^7\), which are distributed in variable geographic regions\(^5\). For instance, genotypes B and C are prevalent in China\(^8\)–\(^9\), whereas genotypes A, D, and E are predominant in Northern Europe, North America and India\(^5\)–\(^6\). Accumulating evidences suggested that infection with genotype C HBV was associated with more aggressive liver disease than with genotype B\(^10\)–\(^14\). However, it remains unknown whether this phenomenon can be generalized to patients with HBV-ACLF. Moreover, previous studies showed that the antiviral response to lamivudine, telbivudine, or adefovir was not different between patients with genotypes B and C CHB\(^15\)–\(^19\). Again, little is known about whether this relationship between HBV genotypes and antiviral efficacy can be generalized to patients with HBV-ACLF, particularly entecavir- or tenofovir-treated patients with HBV-ACLF.

Therefore, we conducted the present study to investigate the impact of HBV genotypes on the clinical and therapeutical outcomes in a cohort of patients with HBV-ACLF who were treated by the currently most potent antiviral therapies, entecavir or tenofovir (tenofovir disoproxil fumarate).

**Patients and Methods**

This study was a prospective, observational cohort study approved by the institutional ethics committee of the Second Affiliated Hospital of Kunming Medical University and conformed to the provisions of the Helsinki Declaration of 1975, as revised in 2008. All enrolled patients provided written informed consents.

**Patients**

A total of 89 antiviral treatment-naïve patients with HBV-ACLF admitted to the Second Affiliated Hospital of Kunming Medical University, China were investigated between January 2015 and January 2018. All cases of ACLF were caused by spontaneous HBV reactivation. Four patients were lost to follow-up within two weeks after the start of the study, who were thus not included in the study analysis due to lack of data at two weeks and at three months. In the end, only 73 consecutive patients were analyzed (Fig. 1). All these patients were aged between 18 and 65 years old who were negative for markers of hepatitis A, C, D, E virus (HAV, HCV, HDV, HEV), or human immunodeficiency virus (HIV) infection, and were free of HCC or any extrahepatic malignancy. None of the patients had the following medical histories: alcohol abuse, hepatotoxic drug use, chronic autoimmune, metabolic, renal, cardiac or pulmonary diseases, previous splenectomy, antiviral treatment or immunosuppressive/cytotoxic therapy during the past 6 months. All patients had HBV genotyping data with genotype B or C.

**Study Design**

73 consecutive patients were stratified into two groups according to HBV genotypes, namely genotype B group \((n = 33)\) and C group \((n = 40)\). All patients were prospectively followed-up every 3–5 days during the hospitalization period, at the 3rd month and subsequently every 3–6 months till death or the study due date (30 January 2018). The follow-up visit at the 3rd month was counted as the first follow-up visit. Clinical evaluation and various laboratory tests and radiographic investigations were conducted at each follow-up visit.

**Laboratory Tests and Radiographic Investigations**

Markers of HAV, HBV, HCV, HDV and HIV (anti-HAV, HBsAg, HBsAb, HBeAg, HBeAb, HbcAb, anti-HCV, anti-HDV, and anti-HIV) infection were assayed at baseline by commercial kits (Abbott Laboratories). HBV DNA loads were measured every 2–4 weeks during the hospitalization period or at each follow-up visit by the use of COBAS AmpliPrep-COBAS TaqMan HBV DNA test (CAP–CTM; Roche Molecular Systems, Inc., Branchburg, NJ, USA) with a lower detection limit of 500 IU/mL. HBV genotypes B and C were determined at baseline by using the real-time fluorescent PCR quantitative analyzer (ABI 7300, USA). Other laboratory tests including complete blood cell (CBC) counts, hepatic and renal functions, electrolytes, blood sugar and lipids, coagulation test were...
performed every 3–5 days during the hospitalization period or at each follow-up visit according to standard operational procedures. All patients were subjected to abdominal ultrasound, computed tomography (CT) and/or magnetic resonance imaging (MRI) scans at baseline or at each follow-up visit.

**Treatment protocol**

All patients took daily entecavir (0.5 mg/day) or tenofovir (300 mg/day) once they were tested positive for HBV-DNA throughout the study period. Most patients also underwent artificial liver support (ALS) treatment by therapeutic plasma exchange (TPE) and/or double plasma molecular absorption system (DPMAS) as described in previous studies.

During the hospitalization period, all patients received standard medical treatment, including intravenous reduced glutathione, polyene phosphatidylcholine, glycyrrhizin, and ademetionine. Intravenous antibiotics were administered when a patient had signs of bacterial infection. Transfusion of human albumin, and other blood constituents such as red blood cells, fresh frozen plasma, and platelets was performed when required. Oral polyene phosphatidylcholine and glycyrrhizin were prescribed to all patients after they were discharged from hospital, which were discontinued when their liver function tests were normal. Oral diuretics and lactulose were also prescribed if required. All adverse events were carefully documented during the study.

**Definitions and diagnosis**

Chronic HBV infection was defined as positivity of hepatitis B surface antigen (HBsAg) for over 6 months. Spontaneous HBV reactivation was defined as an increase in alanine aminotransferase (ALT) level > 5 times upper limit of normal (ULN) along with HBV-DNA level > 10^5 copies/ml (≈1.8 × 10^4 IU/ml) in a patient without any known cause for HBV reactivation. The ACLF was diagnosed when: serum bilirubin ≥ 1.5 or prothrombin time (PT) < 5 mg/dl, an international normalized ratio (INR) ≥ 1.5 or prothrombin activity (PTA) < 40%, complicated within 4 weeks by the development of ascites and/or encephalopathy in a patient with previously detected or undetected chronic liver disease. HBeAg seroconversion was defined as the loss of HBeAg and detectability of HBeAb in the serum samples. Child-Turcotte-Pugh (CTP) and model for end-stage liver disease (MELD) scores were derived from the laboratory data according to the validated formulas to assess the liver function.

The survival period was defined as the interval between the date of recruitment and death or the end of this study (January 30st, 2018), whichever occurred first. Cirrhosis was diagnosed by CT and/or MRI scans, which demonstrated reduced liver size accompanied by disproportional enlargement of the left lobe and shrinkage of the right lobe, irregular liver surface, widened hepatic fissure, coarse liver parenchyma, and indications of portal hypertension (i.e., ascites, splenomegaly, gastroesophageal varices). The diagnosis of HCC was confirmed when a unique tri-phasic feature was present in CT and/or MRI scans.

**Assessment of virological, biochemical and clinical responses**

Virologic response was evaluated by the reduction of HBV-DNA level at 2 weeks, and the percentage of patients achieving undetectable HBV-DNA level at three months. The biochemical response was assessed by the improvements of CTP and MELD scores at two weeks and three months. The clinical response was appraised by the survival rate at three months and adverse events during the study.

**Statistical analysis**

All statistical analyses were performed using the software SPSS version 17.0 for windows (Chicago, IL). Quantitative variables were presented as median (range), and qualitative variables as proportions (frequency). Quantitative and qualitative variables between the two groups were compared by the Mann–Whitney U test and Chi-square test or Fisher’s exact test, respectively. Survival analysis were conducted by Kaplan–Meier method and compared by log-rank test. The independent predictors for mortality were assessed by the univariate and multivariate analyses performed using the Cox regression model. To avoid the problem of overfitting and collinearity, we took the following measures: multivariate analyses included only factors that showed statistical significance with p values < 0.05 in univariate analyses; as CTP score was derived from albumin, prothrombin time (PT), ascites, and MELD score from total bilirubin (TBIL), INR and creatinine (Cr), we included only CTP and MELD scores (excluding albumin, PT, ascites, TBIL, INR and Cr) in multivariate analyses. All P values were 2-tailed and the significance level was set at 0.05.

**Results**

**Baseline Characteristics of the study population**

As shown in Table 1, the demographic and other characteristics of the enrolled patients stratified by HBV genotype were well balanced at baseline. There was no difference in age, sex and cirrhosis ratio, HBeAg positivity, antiviral and ALS treatment, initial CBC counts and liver function test results including CTP and MELD scores from total bilirubin (TBIL), INR and creatinine (Cr), we included only CTP and MELD scores (excluding albumin, PT, ascites, TBIL, INR and Cr) in multivariate analyses. All P values were 2-tailed and the significance level was set at 0.05.
### Table 1 Baseline Characteristics of the study population

| Variables                  | Genotype B (n = 33) | Genotype C (n = 40) | P value |
|---------------------------|---------------------|---------------------|---------|
| Age (yr)                  | 55 (20-65)          | 50 (19-65)          | 0.253   |
| Male, n (%)               | 29 (87.9%)          | 32 (80.0%)          | 0.366   |
| Cirrhosis, n (%)          | 23 (69.7%)          | 27 (67.5%)          | 0.841   |
| HBeAg positivity, n (%)   | 6 (18.2%)           | 14 (35.0%)          | 0.109   |
| HBV DNA (log10 IU/ml)     | 5.67 (4.28-7.72)    | 5.32 (4.27-7.34)    | 0.256   |
| TDF/ETV, n (%)            | 16 (48.5%)/17 (51.5%) | 21 (52.5%)/19 (47.5%) | 0.733   |
| WBC (3.50-9.50 × 10^9/l)  | 6.05 (2.40-17.09)   | 5.38 (2.39-10.53)   | 0.214   |
| HGB (130-175 g/l)         | 124.0 (83-160)      | 122.5 (68-162)      | 0.549   |
| PLT (125-350 × 10^9/l)    | 98 (44-214)         | 95 (44-343)         | 0.786   |
| PT (11.0-15.0 s)          | 19.9 (16.9-29.0)    | 20.9 (17.9-28.6)    | 0.329   |
| INR (0.80–1.30)           | 1.86 (1.51–2.72)    | 1.88 (1.51–2.70)    | 0.517   |
| Albumin (35–50 g/l)       | 29.0 (23.6–37.9)    | 29.4 (21.4–41.2)    | 0.542   |
| ALT (5–40 U/l)            | 261.0 (210–1169)    | 285.5 (124–1124)    | 0.576   |
| AST (8–40 U/l)            | 265.0 (123–1728)    | 286.0 (115–1378)    | 0.575   |
| TBA (0–10 µmol/l)         | 241.0 (102–496.7)   | 212.3 (85.7–610.3)  | 0.346   |
| TBL (3.4–17.1 µmol/l)     | 282.0 (123.1–502.9) | 272.3 (98.6–852.9)  | 0.996   |
| DBIL (0–5.1 µmol/l)       | 237.0 (87.9–430.4)  | 226.3 (58.3–695.4)  | 0.812   |
| Cr (62–115 µmol/l)        | 68 (50–80)          | 68 (55–106)         | 0.714   |
| Aspergillus, n/o/mild/    | 6 (18.2%)/11        | 7 (17.5%)/11        | 0.837   |
| moderate to severe, n (%) | (33.3%)/1648.5%     | (27.5%)/22          | (55.0%) |
| CTP score                 | 11 (8–13)           | 11 (8–14)           | 0.941   |
| MELD score                | 21.0 (16.7–27.5)    | 21.8 (14.4–27.9)    | 0.268   |
| Artificial liver support  |                      |                     | 0.980   |
| No. treatment             | 5 (15.2%)           | 7 (17.5%)           |         |
| TPE, n (%)                | 6 (18.2%)           | 6 (15.0%)           |         |
| DPMA, n (%)               | 15 (45.5%)          | 18 (45.0%)          |         |
| Both TPE and DPMA, n (%)  | 7 (21.2%)           | 9 (22.5%)           |         |
| Hospital stay (day)       | 22.0 (16–36)        | 23.5 (15–46)        | 0.850   |
| Follow-up duration (week) | 11 (6–48)           | 10 (2–48)           | 0.037   |

ALT alanine aminotransferase, AST aspartate aminotransferase, Cr creatinine, CTP Child-Turcotte-Pugh, DPMAS double plasma molecular adsorption system, DBIL direct bilirubin, ETV entecavir, HBeAg hepatitis B e antigen, HBV hepatitis B virus, HGB hemoglobin, INR international normalized ratio, MELD model for end-stage liver disease, PLT platelet, PT prothrombin time, TBA total bile acid, TBL total bilirubin, TDF tenofovir, TPE therapeutic plasma exchange, WBC white blood cell

\*P value, by student’s t test or \*2 test

Virological and serological responses

**HBV-DNA change at two weeks and at three months**

After two weeks of antiviral therapy with entecavir or tenofovir, HBV-DNA level in genotype B group was significantly lower than in genotype C group (median and range: 3.04: 2.70–5.01 vs. 3.34: 2.70–4.06; P = 0.005; Fig. 2a), and HBV-DNA reduction was significantly greater in genotype B group than in genotype C group (median and range: 2.48:1.21–4.32 vs. 1.89:1.12–3.68; P = 0.026; Fig. 2b). At 2 weeks, 12 patients in genotype B group (36.4%; 12/33) had undetectable HBV-DNA level, which was markedly greater than in genotype C group (10.0%; 4/40) (P = 0.007; Fig. 2c). At three months, HBV-DNA was undetectable in all survived patients in the genotype B and C groups (data not shown).

**HBeAg loss and seroconversion at two weeks and at three months**

Of the HBeAg positive patients at baseline, two of six patients in genotype B group as compared to none of 14 in genotype C group experienced HBeAg loss at 2 weeks (2/6 vs. 0/14; P = 0.079; Fig. 2d). Of the survived patients with positive HBeAg, two of two patients in genotype B group as compared to none of three in genotype C group showed HBeAg loss at three months (2/2 vs. 0/3; P = 0.100; Fig. 2e). HBeAg seroconversion was not observed in any patient.

**Biochemical response**

After two weeks of treatment, CTP (median and range: 10.8–14 vs. 12.7–13; P = 0.032) and MELD (median and range: 17.4:12.4–26.4 vs. 20.6:12.2–28.5; P = 0.039) scores were significantly lower in genotype B group than in C group. However, CTP and MELD scores were comparable between the genotype B and C groups at three months (Fig. 3a, b), which were significantly improved compared to at baseline (data not shown). Similarly, CTP (median and range: 10.7–13 vs. 12.9–14; P = 0.000) and MELD (median and range: 17.1:13.1–26.4 vs. 22.2:12.2–28.5; P = 0.000) scores were significantly improved in patients with HBV-DNA reduction ≥2log10 IU/ml (n = 44) compared to those with HBV-DNA reduction <2log10 IU/ml (n = 29) at 2 weeks (Table 2). In patients with HBV-DNA reduction ≥2log10 IU/ml, CTP (median and range: 10.7–13 vs. 11.8–13; P = 0.296) and MELD (median and range: 17.1:13.1–26.4 vs. 21.0:14.4–27.9; P = 0.000) scores were decreased at 2 weeks compared to at baseline. In contrast, CTP (median and range: 12.9–14 vs. 12.8–14; P = 0.011) and MELD (median and range: 22.2:12.2–28.5 vs. 21.9:18.7–27.7; P = 0.703) scores at two weeks were increased in those with HBV-DNA reduction <2log10 IU/ml, when compared to at baseline (Table 2).
Overall survival rate at three months
At three months, a total of 35 patients died. All deaths were related to liver failure progression or complications, including sepsis, severe hepatic encephalopathy and multiple organ failure. The overall survival rate was significantly higher in the genotype B group than in C group (64.4% vs. 38.4%; $P = 0.013$; Fig. 4).

Independent predictors for mortality at three months
As shown in Table 3, only variables at baseline and at two weeks significantly associated with mortality in the univariate analysis were included in the multivariate analysis. Compared to genotype B, genotype C was associated with 2.138-fold increased risk for mortality ($P = 0.041$). The MELD score at two weeks was also significantly associated with mortality (Hazard ratio [HR]: 1.664, 95% confidence interval [CI]: 1.077–2.571; $P = 0.022$). In contrast, HBV-DNA decline $\geq 2\log_{10}$ IU/ml at two weeks was associated with 0.225-fold (or 77.5%) decreased risk for mortality when compared to HBV-DNA decline $< 2\log_{10}$ IU/ml ($P = 0.016$).

Adverse events
During the study, one patient developed pseudoaneurysm related to the placement of femoral vein catheter for ALS procedure (genotype B vs. C: 0/33 vs. 1/...
40; \( P = 0.360 \)). Four patients were allergic to plasma during the TPE procedure (genotype B vs. C: 2/33 vs. 2/40; \( P = 0.792 \)). Three patients experienced mild nasal or gingival bleeding after ALSS procedure (genotype B vs. C: 1/33 vs. 2/40; \( P = 0.673 \)). All these events were successfully managed by conservative methods. None of the patients experienced allergy to antiviral agents or the reported side effects related to entecavir or tenofovir.

**Discussion**

To our best knowledge, the present study is the first one that demonstrated the association between HBV genotype and antiviral efficacy and clinical outcomes in patients with HBV-ACLF. Clearly, our study showed that patients with genotype B HBV-ACLF had faster antiviral response and better biochemical response at two weeks, and improved overall survival at three months compared to those with genotype C HBV-ACLF, though the serological response was similar between the two groups. In addition, our study identified genotype, MELD score and HBV-DNA decline at two weeks as three independent predictors for mortality at three months.

ACLF is a severe liver disease with extremely high 1-month and 3-month mortalities\(^4,20–22\) that can be reduced by ALSS therapy\(^21,24\), and antiviral therapy for HBV infection\(^22,25\). Thus, in this study all patients received antiviral therapy for HBV infection, and over 80% of patients underwent ALSS therapy, though the interaction between these two types of therapies was largely unknown. Intriguingly, the association between HBV genotype and antiviral response to interferon or NAs has been investigated. Previous studies indicated that the response to conventional or pegylated interferon therapy was better in genotype B than in genotype C patients with CHB\(^26–30\). Despite the antiviral action was different between NAs and interferon, our study showed that patients with genotype B HBV-ACLF had greater HBV-DNA reduction and higher proportion of patients

| Table 2 | Changes of CTP or MELD scores in patients with different HBV-DNA declines at two weeks |
|---------|----------------------------------------------------------------|
|         | \(<2\log_{10} \text{IU/ml} (n = 29)\) | \(\geq 2\log_{10} \text{IU/ml} (n = 44)\) | \(P\) value |
| CTP score at baseline | 12 (8–14) | 11 (8–13) | 0.366 |
| CTP score at 2 weeks | 12 (9–14)* | 10 (7–13)$ | 0.000 |
| MELD score at baseline | 21.9 (18.7–27.5) | 21.0 (14.4–27.9) | 0.149 |
| MELD score at 2 weeks | 22.2 (12.6–28.5)$ | 17.1 (13.1–26.4)** | 0.000 |

CTP child-turcotte-pugh score, HBV hepatitis B virus, MELD model of end-stage liver disease

*\(P > 0.05\)  \$\(P < 0.05\)  **\(P < 0.001\) compared to at baseline
achieving undetectable HBV-DNA after two weeks of entecavir or tenofovir treatment, which was consistent with the previous studies.\(^{26-30}\) However, conflicting results were observed in patients treated by other less potent oral NAs. For example, several studies reported that lamivudine seemed to result in a better virological response in genotype B than genotype C patients with CHB\(^{31,32}\), whereas other studies suggested that the response to lamivudine was not different between the two groups.\(^{15,16,18,33,34}\) Moreover, previous studies also demonstrated that the response to telbivudine or adefovir was not different between the two groups of patients.\(^{18,19,35,36}\) Currently, the exact mechanism underlying the relationship between HBV genotypes and antiviral response remains unclear. Nonetheless, the antiviral efficacy is influenced by viral, host, or environmental factors in patients with chronic HBV infection, which may account for the discrepant results between our study and the previous ones, because the host immunologic disposition, the impact of more potent antiviral therapy on host and virus, and the host-viral interaction differed between our study and the previous studies.

Previous studies indicated that genotype B HBV was associated with a higher rate of HBeAg seroconversion compared to genotype C HBV after interferon treatment.\(^{27,28}\) In contrast to this finding, another study suggested that HBeAg seroconversion was not different between genotype B and C HBV after lamivudine treatment.\(^{31}\) In our study, genotype B group tended to have higher rate of HBeAg loss compared to genotype C group at two weeks, and the rates of HBeAg loss or seroconversion were not different between the two groups at three months. However, reliable conclusion regarding HBeAg loss or seroconversion in patients with HBV-ACLF can not be drawn from our study, because our study was limited by small sample size and short observation period.

For patient with CHB, previous studies suggested that rapid suppression of HBV-DNA was associated with a greater chance of achieving antiviral response and a lower risk of genotypic resistance.\(^{38,39}\) For patients with HBV-ACLF, Garg et al. showed that rapid suppression of HBV-DNA in patients treated by tenofovir was associated with improved CTP and MELD scores at 45 and 90 days compared to those treated by placebo.\(^{22}\) In line with these studies, our study showed that patients with greater HBV-DNA reduction (\(\geq 2\log_{10}\) IU/ml) had significantly improved CTP and MELD scores at two weeks, when compared to those with lower HBV-DNA reduction (<2log_{10} IU/ml) (Table 2). In addition, our study also showed that genotype B group had significantly improved lower CTP and MELD scores as compared to than genotype C group at two weeks, which may be associated with the much rapider suppression of HBV-DNA in genotype B group than in genotype C group (Fig. 2b).

In our study, genotype B group had significantly higher overall survival rate at three months compared to genotype C group, which can be explained by the rapider viral response and more improved liver function at two weeks achieved in genotype B group than in C group. However, the exact reasons for the more aggressive disease course in genotype C HBV-ACLF are still unclear. Previous studies indicated that genotype B HBV compared to genotype C HBV was associated with an earlier and higher rate of spontaneous HBeAg seroconversion, which might explain the less aggressive liver disease in patients with genotype B.\(^{40}\) Moreover, previous studies showed that basic core promoter (BCP) mutation might increase the risk of liver disease progression, and infection by genotype C HBV had a higher prevalence of BCP mutation than those infected by genotype B HBV.\(^{41}\) Previous studies also showed that core promoter mutation was implicated in more severe liver disease, and TA core promoter mutation was more prevalent in genotype C than B HBV.\(^{42}\) These findings may further explain why genotype C HBV-ACLF had a more aggressive disease course than genotype B.

In our study, multivariate analyses revealed that genotype was significantly associated with mortality at three months, which further supported our conclusion that
genotype B was associated with better clinical outcome than genotype C in patients with HBV-ACLF. Consistent with the study by Garg et al.\textsuperscript{22}, our study also identified HBV-DNA decline at two weeks as an independent predictor for mortality at three months. Moreover, our study showed that MELD score at two weeks was significantly associated with mortality at three months, suggesting improvement of liver function (with low CTP and MELD scores) at two weeks was a critical determinant for survival at three months.

Our study had several limitations. First, the sample size was small and the follow-up period was relatively short. Second, the treatment allocation was not randomized. However, the treatment allocation, including ALSS and antiviral treatment, was well balanced between the two groups, and our study was a prospective one that firstly compared the therapeutical and clinical outcomes in genotype B and C HBV-ACLF patients who were treated by currently the most potent antiviral agents, which provided valuable information to clinicians.

In conclusion, our study demonstrated that genotype B was associated with higher short-term antiviral response and survival rate than genotype C in patients with HBV-ACLF. The genotype, MELD score and HBV-DNA

| Variables at baseline |
|-----------------------|
| Genotype\textsuperscript{a} | 2.327 | 1.137–4.764 | 0.001 |
| Cirrhosis\textsuperscript{b} | 2.600 | 1.130–5.980 | 0.025 |
| HBV-DNA (log\textsubscript{10} IU/ml) | 0.469 | 0.305–0.724 | 0.001 |
| PLT (x 10\textsuperscript{9}/l) | 0.994 | 0.987–1.000 | 0.048 |
| Creatinine (µmol/l) | 1.065 | 1.019–1.114 | 0.006 |
| Ascites\textsuperscript{c} | 1.736 | 1.039–2.900 | 0.035 |
| Antiviral therapy\textsuperscript{d} | 0.480 | 0.241–0.955 | 0.036 |
| MELD score | 1.210 | 1.035–1.415 | 0.017 |

| Variables at two weeks |
|------------------------|
| MELD score | 1.595 | 1.383–1.840 | 0.000 |
| HBV-DNA reduction (log\textsubscript{10} IU/ml)\textsuperscript{e} | 0.024 | 0.003–0.179 | 0.000 |
| WBC (x 10\textsuperscript{9}/l)\textsuperscript{f} | 3.399 | 1.731–6.674 | 0.000 |
| PLT (x 10\textsuperscript{9}/l) | 0.990 | 0.983–0.998 | 0.015 |
| PT (s) | 1.302 | 1.176–1.441 | 0.000 |
| INR | 11.996 | 4.984–28.873 | 0.000 |
| Albumin (g/l) | 0.819 | 0.733–0.916 | 0.000 |
| TBIL (µmol/l) | 1.008 | 1.006–1.011 | 0.000 |
| DBIL (µmol/l) | 1.009 | 1.006–1.012 | 0.000 |
| Creatinine (µmol/l) | 1.054 | 1.019–1.090 | 0.002 |
| Ascites\textsuperscript{c} | 6.834 | 2.795–16.706 | 0.000 |
| CTP score | 2.356 | 1.724–3.218 | 0.000 |
| HBV-DNA (log\textsubscript{10} IU/ml) | 3.044 | 1.542–6.012 | 0.001 |

CI confidence interval, CTP child-turcotte-pugh, DBIL direct bilirubin, HBV hepatitis B virus, HR hazard ratio, INR international normalized ratio, MELD model for end-stage liver disease, PLT platelet, PT prothrombin time, TBIL total bilirubin, WBC white blood cells

\textsuperscript{a}Genotype: genotype B = 1, genotype C = 2

\textsuperscript{b}Cirrhosis: no = 0, yes = 1

\textsuperscript{c}Ascites: none = 0, mild = 1, moderate to severe = 2

\textsuperscript{d}Antiviral therapy: entecavir = 1, tenofovir = 2; WBC (x 10\textsuperscript{9}/l)

\textsuperscript{e}HBV-DNA reduction (log\textsubscript{10} IU/ml): < 2 log\textsubscript{10} IU/ml = 1, ≥ 2 log\textsubscript{10} IU/ml = 2

\textsuperscript{f}WBC (x 10\textsuperscript{9}/l): ≤ 9.5 x 10\textsuperscript{9}/l = 1, > 9.5 x 10\textsuperscript{9}/l = 2
decline at two weeks were independent predictors for mortality at three months. More studies with larger sample size and longer follow-up period are warranted to confirm our results.

Study Highlights

WHAT IS CURRENT KNOWLEDGE
- Genotype C HBV was associated with more aggressive liver disease than with genotype B.
- Response to lamivudine, telbivudine, or adefovir was similar between genotypes B and C CHB.
- Interferon therapy was better in genotype B than in genotype C patients with CHB.

WHAT IS NEW HERE
- demonstrating that genotype B was associated with better viral response at two weeks than genotype C in HBV-ACLF;
- showing that genotype B was associated with more improved liver function than genotype C at two weeks in HBV-ACLF;
- illustrating that genotype B was associated with higher 3-month overall survival than genotype C in HBV-ACLF;
- identifying genotype, MELD score, and HBV-DNA decline at two weeks as the independent predictors for 3-month mortality.

TRANSLATIONAL IMPACT
- Genotype is very important for predicting the antiviral response and short-term outcome in patients with HBV-ACLF. For patients with genotype C HBV-ACLF, early and more aggressive therapy, such as liver transplantation, is justified.

Competing interests
Conflict of interest: The authors declare that they have no conflict of interest.
Guarantor of article: Yue-Meng Wan is the guarantor of this article, accepting full responsibility for the conduct of the study, having access to the data and control of the decision to publish.
Specific author contributions: Yue-Meng Wan: planning and conducting the study; managing patients; collecting and interpreting data; drafting manuscript. He has approved the final draft submitted. Yu-Hua Li: conducting the study; managing patients; collecting data. She has approved the final draft submitted. Xi-Nan Wu: planning the study; interpreting data. He has approved the final draft submitted. Ying Xu: planning and conducting the study; interpreting data. She has approved the final draft submitted. Hua-Mei Wu: conducting the study; managing patients; collecting data. She has approved the final draft submitted. Yue-Meng, W. et al. The effect of plasma exchange on entecavir-treated CHB patients with acute-on-chronic liver failure. J. Viral Hepat. 20, 269–282 (2013).

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