The relationship between zinc deficiency and infectious complications in patients with hepatitis B virus-related acute-on-chronic liver failure

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Abstract

Background  The prevalence of zinc deficiency is high in patients with chronic liver disease, but few studies have hitherto explored the relationship between the serum zinc level and hepatitis B virus (HBV)-related acute-on-chronic liver failure (ACLF). This study aimed to assess the association between zinc deficiency and infectious complications, and model for end-stage liver disease (MELD) score in patients with HBV-related ACLF.

Methods  Patients with HBV-related ACLF from the Department of Infectious Diseases of the Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) between January 2019 and December 2019 were retrospectively analysed in this study. Their demographic, clinical, and laboratory data were retrieved from the hospital information system and analysed. The Student’s t-test was used for normally distributed continuous variables between two groups and the Chi-square test was used for categorical data. Univariate and multivariate logistic regression analyses were applied to identify independent parameters.

Results  A total of 284 patients were included in this study, including 205 liver cirrhosis and 79 non-cirrhosis patients. The proportion of patients with zinc deficiency was the highest (84.5%), followed by subclinical zinc deficiency (14.1%) and normal zinc level (1.4%). Patients in the zinc deficiency group had a higher MELD score than the subclinical zinc deficiency or normal zinc group (P = 0.021). Age, total bilirubin, and serum zinc level were independent factors for infection (P < 0.05). The serum zinc level in patients without complications at admission was significantly higher than that in patients with complications (P = 0.004). Moreover, the serum zinc level in patients with prothrombin time activity (PTA) of <20%
was significantly lower than that in patients with \(20\% \leq \text{PTA} < 30\%\) \((P = 0.007)\) and that in patients with \(30\% \leq \text{PTA} < 40\%\) \((P < 0.001)\).

**Conclusions**  Zinc deficiency is common in patients with HBV-related ACLF. Zinc deficiency is closely associated with infectious complications and MELD score in patients with HBV-related ACLF.

**Key words:** hepatitis B virus; acute-on-chronic liver failure; mortality; zinc; complications

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### Introduction

Current evidence suggests that hepatitis B virus (HBV) infects >2 billion people worldwide [1]. According to the World Health Organization, an estimated 257 million people have chronic HBV infection globally [2]. In China, 20–30 million people reportedly have chronic hepatitis B (CHB) [3]. It has been established that HBV-related acute-on-chronic liver failure (ACLF) is the most common cause of liver failure in China [4]. In this respect, Xie et al. [5] found that HBV activation and withdrawal of antiviral drugs were the most common causes of HBV-related ACLF in southwest China. An extremely high 90-day mortality rate was observed attributed to the lack of effective therapies for patients with HBV-related ACLF [6-8]. Currently, liver transplantation remains the only curative approach for ACLF, but this process is associated with serious problems, such as graft shortage, surgical complications, organ rejection, and high cost. Accordingly, much emphasis has been placed on early intervention and comprehensive treatment of this patient population [9].

It is widely acknowledged that zinc is a catalytic, structural (zinc fingers), and regulatory ion, playing a pivotal role in many biological functions, including immune responses [10]. Interestingly, zinc can affect both innate and adaptive immune cells; it regulates macrophage function, which plays a key role in innate immunity by regulating host defense responses [11]. It has been shown that zinc homeostasis is crucial for the normal function of the immune system [12]. Current evidence suggests zinc deficiency is related to several infectious diseases such as malaria, human immunodeficiency virus (HIV), tuberculosis, measles, and pneumonia [13]. Zinc may possess potent antiviral effects mediated by zinc-binding proteins such as metallothioneins in many viruses [14]. An in vitro study substantiated that zinc could inhibit hepatitis C virus (HCV) replication [15]. It is widely thought that increased zinc levels in children with chronic HBV infection indicate a positive response to interferon treatment [16]. An increasing body of evidence suggests that zinc deficiency is common in patients with chronic liver disease, particularly in cirrhotic patients with Child–Pugh class B or C and with a model for end-stage liver disease (MELD) score of \(\geq 15\) [17, 18]. Besides, a low serum zinc level has been documented in patients with fulminant and subacute hepatic failure [19].

Many prognostic scoring systems have been documented in the literature for evaluating the severity of ACLF [7, 20–22]. Collectively, the indicators in these scoring systems are mostly related to liver functions, such as serum total bilirubin (TBL), prothrombin time–international normalized ratio (PT–INR), serum creatinine, and hepatic encephalopathy (HE); however, no scoring system has included the serum zinc level. The association between the serum zinc level and HBV infection or HBV-related ACLF has been understudied. Therefore, this study aimed to explore the relationship between zinc deficiency and infectious complications and MELD score in patients with HBV-related ACLF.

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### Patients and methods

#### Study design and participants

This retrospective study included patients diagnosed with HBV-related ACLF and treated at the Department of Infectious Diseases of the Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) between January 2019 and December 2019.

The inclusion criteria for this study were based on the consensus recommendations for ACLF from Asian Pacific Association for the Study of the Liver (APASL) [9] and the guidelines for the diagnosis and treatment of liver failure in China [4]. The inclusion criteria were as follows: (i) hepatitis B virus surface antigen (HBsAg)-positive or HBV deoxyribonucleic acid (DNA)-positive for >6 months; (ii) age between 18 and 65 years; (iii) serum creatinine > 20% or PT–INR of >1.5.

The exclusion criteria were as follows: (i) patients with hepatocellular carcinoma or other malignancy; (ii) pregnant or lactating females; (iii) patients with HIV infection or congenital immune deficiency diseases; (iv) patients with uncontrolled diabetes or autoimmune diseases; (v) patients with a history of organ transplantation or with dysfunctions not related to liver disease; or (vi) incomplete data or loss to follow-up.

This study was conducted in accordance with the Ethical Guidelines of the 1975 Declaration of Helsinki. The study protocol was approved by the Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (approval no. 202002–009–01). Given the retrospective nature of our study, the need for informed consent was waived.

#### Data collection

The demographic, clinical, and laboratory data of all included patients upon admission were collected from the hospital information system. The demographic data included age and sex. Clinical data included pre-existing chronic liver diseases, complications, treatment, and treatment outcomes. Pre-existing chronic liver diseases included hepatitis and cirrhosis. Complications of ACLF included ascites, HE, hepatorenal syndrome (HRS), gastrointestinal bleeding (GIB), and infection. Infectious complications were diagnosed based on infection-related symptoms, signs, laboratory indicators, or imaging and were recorded throughout the hospitalization course. Treatment outcomes included recovery and unfavorable events such as death, liver transplantation, and treatment abandonment. Laboratory data included blood cell routine (white blood cell count, platelets [PLT] count, serum hemoglobin level), biochemical parameters (serum aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase [GGT], albumin [ALB], TBL, direct bilirubin, total bile acid, cholesterol [CHE], total cholesterol [TC], sodium, zinc, copper, magnesium, blood urea nitrogen, and creatinine levels), coagulation function (prothrombin time [PT], PTA, PT–INR), virological markers (HBsAg, HBV DNA), tumor markers (alpha-fetoprotein,
ferritin), abdominal ultrasound, or computed tomography. The MELD score was calculated using the following formula:
MELD = 9.57 \times \log_{e} \text{creatinine} \ (\text{mg/dL}) + 3.78 \times \log_{e} \text{TBIL} \ (\text{mg/dL}) + 11.20 \times \log_{e} \text{PT–INR} + 6.43 \ [23].

Zinc deficiency definition and serum zinc examination
There is no definite standard for zinc deficiency in China. Given that zinc is an essential ion element and the patients enrolled in this study were Chinese in East Asia, we chose the Japanese Society of Clinical Nutrition criteria, which defines a serum zinc level of <60 \mu{g}/dL (9.18 \mu{mol}/L) as zinc deficiency, 60–80 \mu{g}/dL (9.18–12.24 \mu{mol}/L) as serum zinc level <80 \mu{g}/dL (12.24 \mu{mol}/L) as subclinical zinc deficiency, and 80–130 \mu{g}/dL (19.89–31.74 \mu{mol}/L) as normal zinc range [24], from Japan, which is a neighboring country also in East Asia. Given that Chinese and Japanese are East Asians, the criteria are precise and suitable for the study population.

Blood samples from patients were collected at 7 a.m. on the second day of admission and transferred to the laboratory. The serum concentration of zinc was assessed using an atomic absorption spectrophotometer (Leadman Biochemistry, Beijing, China).

Statistical analysis
Continuous data with normal distribution are expressed as mean ± standard deviation, whereas continuous data with abnormal distribution are expressed as median (interquartile range). Categorical data are presented as count and percentage (%). The significance of differences for normally distributed continuous variables between two groups was assessed using the Student’s t-test; for multiple groups (more than two), one-way Analysis of Variance (ANOVA) was used. The Mann–Whitney test was used to analyse the differences in continuous variables with a non-parametric distribution and the Chi-square test was used for categorical data. Univariate and multivariate logistic regression analyses were applied to identify independent parameters linked with infectious complications. The statistical significance level for all tests was set at a P-value of <0.05. Statistical analyses were conducted using SPSS V23.0 (SPSS Statistics V23, IBM Corporation, Somers, New York, USA).

Results
Patient enrollment and baseline characteristics
A total of 466 patients with ACLF were screened in this study and 402 patients with HBV-related ACLF were identified. Finally, 284 patients with complete data were included in this study (Figure 1), including 245 males and 39 females. Liver cirrhosis was observed in 72.2% of patients (n = 205). The proportion of patients with zinc deficiency was the highest (84.5%), followed by subclinical zinc deficiency (14.1%) and normal zinc level (1.4%). The incidence of zinc deficiency in patients with non-cirrhosis, compensated cirrhosis, and decompensated cirrhosis were 75.9% (60/79), 84.0% (21/25), and 88.3% (159/180), respectively. Subsequently, patients were divided into the zinc deficiency group (n = 240) and the subclinical zinc deficiency or normal zinc group (n = 44). The serum levels of ALB (P = 0.001), GGT (P = 0.007), CHE (P = 0.030), and TC (P = 0.017) were significantly higher in the subclinical zinc deficiency or normal zinc group than in the zinc deficiency group. The zinc deficiency group had a significantly lower proportion of non-cirrhosis patients (P = 0.013) and a higher proportion of patients with decompensated cirrhosis (P = 0.019) than the subclinical zinc deficiency or normal zinc group. The patients in the zinc deficiency group had a significantly higher MELD score than those in the subclinical zinc deficiency or normal zinc group (P = 0.021). All patients received nucleos(t)ide analogs for anti-HBV therapy after admission, including entecavir, tenofovir disoproxil fumarate, tenofovir alafenamide, and other regimens without zinc supplementation after admission. Patients were treated with antibiotics after infectious complications were diagnosed, but no antibiotic prophylaxis treatment was given. The baseline patient characteristics are shown in Table 1.

Prognosis and associated factors
The prognosis for patients was based on treatment outcomes, which were divided into recovery and unfavorable events (death, liver transplantation, and treatment abandonment) groups. The baseline patient characteristics in Table 1 were analysed to identify the factors associated with prognosis. During univariate logistic regression analysis, significant differences in age (P < 0.001), HE (P < 0.001), ascites (P = 0.014), HRS (P = 0.009), infection (P < 0.001), TBIL (P < 0.001), PT–INR (P < 0.001), PLT count (P = 0.019), and MELD score (P < 0.001) were observed between the two groups (Table 2). These variables were then incorporated for multivariate regression. Multivariate logistic regression analysis indicated that HE (P < 0.001), infection (P = 0.017), and MELD score (P < 0.001) were independent factors that influenced prognosis (Table 2).

Serum zinc and infectious complications
The serum zinc level in HBV-related ACLF patients with infectious complications upon admission was significantly higher than that in patients without infectious complications (P = 0.038). The most common infectious complications were peritonitis, pneumonia, and infectious diarrhea; however, there were no significant differences in the serum zinc levels among the patients with these three infections (P = 0.267).
logistic regression analyses. We found that patients with age of 45 years, a TBIL level of > 293 μmol/L, and a serum zinc level of < 3.7 μmol/L were more likely to have infectious complications.

Interestingly, when the number of infectious complications increased from one to three, a gradual downward trend in serum zinc levels was observed, although the difference was not statistically significant (P = 0.214) (Table 3).

We analysed the association between various biochemical parameters presented in Table 1 and infectious complications in HBV-related ACLF patients (Table 4). Univariate logistic regression analysis revealed that age (P = 0.012), ALB level (P = 0.036), TBIL level (P = 0.009), PT (P = 0.038), and serum zinc level (P = 0.007) were prognostic factors. Furthermore, age (P = 0.010), TBIL level (P = 0.009), and serum zinc level (P = 0.035) were identified as independent predictors in the multivariate logistic regression analyses. We found that patients with age of > 51 years, a TBIL level of > 293 μmol/L, and a serum zinc level of < 3.7 μmol/L were more likely to have infectious complications.

### Table 1. The baseline patient characteristics

| Characteristic                          | Overall (n = 284) | Zn deficiency (n = 240) | Subclinical Zn deficiency or normal Zn (n = 44) | P-value |
|----------------------------------------|-------------------|------------------------|-----------------------------------------------|---------|
| Age (years)                            | 45 ± 10           | 45 ± 11                | 45 ± 10                                       | 0.721   |
| Sex, male, n (%)                       | 245 (86.3%)       | 204 (85.0%)            | 41 (93.2%)                                    | 0.147   |
| Anti-HBV therapies, n (%)              | ETV (73.9%)       | 176 (73.3%)            | 34 (77.3%)                                    | 0.924   |
|                                       | TDF (44.4%)       | 36 (15.0%)             | 5 (11.4%)                                     |         |
|                                       | TAF (8.8%)        | 21 (8.8%)              | 4 (9.1%)                                      |         |
|                                       | Others (2.8%)     | 7 (2.9%)               | 1 (2.3%)                                      |         |
| WBC (×10^9/L)                          | 7.8 ± 5.7         | 7.8 ± 3.6              | 7.8 ± 4.4                                     | 0.984   |
| HBG (g/L)                              | 117 ± 22          | 116 ± 22               | 121 ± 27                                      | 0.247   |
| PLT (×10^9/L)                          | 123 ± 66          | 121 ± 63               | 131 ± 83                                      | 0.358   |
| AST (U/L)                              | 213 (103–565)     | 211 (103.3–513.3)      | 272 (156.0–1,033.0)                           | 0.166   |
| ALT (U/L)                              | 308 (73–894)      | 271 (73.0–809.8)       | 616 (91.0–1,290.0)                            | 0.064   |
| ALB (g/L)                              | 35.1 ± 5.8        | 34.5 ± 5.5             | 38.2 ± 5.3                                    | < 0.001 |
| TBIL (μmol/L)                          | 360 ± 137         | 364 ± 130              | 342 ± 170                                     | 0.332   |
| DBIL (μmol/L)                          | 212 ± 93          | 214 ± 88               | 202 ± 119                                     | 0.553   |
| GGT (U/L)                              | 99 ± 63           | 95 ± 59                | 123 ± 77                                      | 0.007   |
| TBA (μmol/L)                           | 250 ± 92          | 252 ± 92               | 242 ± 97                                      | 0.517   |
| Cholinesterase (U/L)                   | 4,096 ± 1,096     | 4,003 ± 1,673          | 4,606 ± 1,747                                 | 0.030   |
| PT (s)                                 | 26.7 ± 8.0        | 27.1 ± 7.9             | 24.6 ± 8.1                                    | 0.055   |
| PT–INR                                 | 2.5 ± 1.0         | 2.5 ± 1.0              | 2.2 ± 1.0                                     | 0.058   |
| BUN (mmol/L)                           | 3.8 (2.6–5.3)     | 3.8 (2.7–5.2)          | 3.4 (2.4–5.6)                                 | 0.603   |
| Creatinine (μmol/L)                    | 80 ± 52           | 80 ± 50                | 80 ± 63                                       | 0.993   |
| TC (mmol/L)                            | 2.7 ± 1.0         | 2.7 ± 1.0              | 3.0 ± 1.1                                     | 0.017   |
| Serum Na (mmol/L)                      | 136.6 ± 4.5       | 136.3 ± 4.1            | 138 ± 5.9                                     | 0.083   |
| Serum Cu (μmol/L)                      | 15.4 ± 5.1        | 12.9 ± 5.1             | 14.5 ± 4.9                                    | 0.065   |
| Serum Mg (mmol/L)                      | 0.8 ± 0.1         | 0.8 ± 0.1              | 0.8 ± 0.1                                     | 0.116   |
| Ferritin (ng/mL)                       | 2,438 ± 1,578     | 2,452 ± 1,578          | 2,340 ± 1,606                                 | 0.730   |
| AFP (ng/mL)                            | 132 ± 46          | 132 ± 45               | 134 ± 49                                      | 0.775   |
| Non-cirrhosis, n (%)                   | 79 (27.8%)        | 60 (25.0%)             | 19 (43.2%)                                    | 0.013   |
| Compensated cirrhosis, n (%)           | 25 (8.8%)         | 21 (8.8%)              | 4 (9.1%)                                      | 0.942   |
| Decompensated cirrhosis, n (%)         | 180 (63.4%)       | 159 (66.3%)            | 21 (47.7%)                                    | 0.019   |
| Complication, n (%)                    | HE (27.8%)        | 71 (30.0%)             | 8 (18.2%)                                     | 0.121   |
| Ascites                                | 186 (65.5%)       | 160 (78.0%)            | 26 (59.1%)                                    | 0.331   |
| HRS                                    | 11 (3.9%)         | 10 (4.2%)              | 1 (2.3%)                                      | 0.549   |
| GIB                                    | 8 (2.5%)          | 7 (2.9%)               | 1 (2.3%)                                      | 0.812   |
| Infection                              | 177 (62.3%)       | 153 (63.8%)            | 24 (54.5%)                                    | 0.247   |
| MELD score                             | 24.9 ± 5.7        | 25.3 ± 5.6             | 23.1 ± 5.9                                    | 0.021   |
| Unfavorable outcomes, n (%)            | 115 (40.5%)       | 99 (41.3%)             | 16 (36.4%)                                    | 0.544   |

Zn, zinc; HBV, hepatitis B virus; ETV, entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; WBC, white blood cell; HGB, hemoglobin; PLT, platelet; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; TBIL, total bilirubin; DBIL, direct bilirubin; GGT, gamma-glutamyl transpeptidase; TBA, total bile acid; PT, prothrombin time; PT–INR, prothrombin time–international normalized ratio; BUN, blood urea nitrogen; TC, total cholesterol; Na, sodium; Cu, copper; Mg, magnesium; AFP, alpha fetal protein; HE, hepatic encephalopathy; HRS, hepatic renal syndrome; GIB, gastrointestinal bleeding; MELD, model for end-stage liver disease; Unfavorable outcome, death, liver transplantation, and treatment abandonment.

### Serum zinc level and other complications

HE, ascites, GIB, and HRS were the other main complications in HBV-related ACLF patients. Patients without complications upon admission had a significantly higher serum zinc level than patients with complications (7.2 ± 2.3 vs 6.5 ± 2.0 μmol/L, P = 0.004). With respect to the number of complications, there was no significant difference in the serum zinc levels between patients with at least two complications and those with fewer than two complications (6.2 ± 2.3 vs 6.6 ± 1.9 μmol/L, P = 0.423).

### Serum zinc level and PTA

The serum zinc levels in HBV-related ACLF patients with coagulation disorders showed a gradual downward trend with the decrease in PTA. Patients with 30% < PTA < 40% had the...
Table 2. Univariate and multivariate logistic regression analysis of prognosis

| Variable   | Univariate analysis | P-value | Multivariate analysis | P-value |
|------------|---------------------|---------|-----------------------|---------|
|            | OR (95% CI)         |         | OR (95% CI)           |         |
| Age        | 1.045 (1.020–1.070) | <0.001  |                       |         |
| HE         | 0.163 (0.090–0.295) | <0.001  | 0.251 (0.132–0.476)   | <0.001  |
| Ascites    | 0.524 (0.312–0.880) | 0.014   | 0.284 (0.157–0.516)   | 0.001   |
| HRS        | 0.063 (0.008–0.495) | 0.009   | 0.055 (0.023–0.131)   | <0.001  |
| Infection  | 0.367 (0.218–0.618) | <0.001  | 0.489 (0.271–0.881)   | 0.017   |
| TBIL       | 0.995 (0.993–0.997) | <0.001  | 0.962 (0.960–0.965)   | <0.001  |
| PT–INR     | 0.313 (0.214–0.458) | <0.001  |                       |         |
| PLT        | 1.005 (1.001–1.009) | 0.019   | 0.998 (0.996–1.000)   | <0.001  |
| MELD score | 0.892 (0.806–0.990) | <0.001  | 0.892 (0.840–0.947)   | <0.001  |

OR, odds ratio; CI, confidence interval; HE, hepatic encephalopathy; HRS, hepatic renal syndrome; TBIL, total bilirubin; PT–INR, prothrombin time–international normalized ratio; PLT, platelet; MELD, model for end-stage liver disease.

Table 3. Serum zinc level in patients with/without infectious complications

| Infection                | No. of patients (%) | Serum zinc level (μmol/L) | P-value |
|--------------------------|---------------------|---------------------------|---------|
| With or without infectious complications |                      |                           | 0.038   |
| Without infection        | 107 (37.7%)         | 7.29 ± 2.25               |         |
| With infection           | 177 (62.3%)         | 6.73 ± 2.17               |         |
| Type of infectious complication |                      |                           | 0.267   |
| Pneumonia                | 127 (71.8%)         | 6.74 ± 2.28               |         |
| Peritonitis              | 114 (64.4%)         | 6.61 ± 1.92               |         |
| Infectious diarrhea      | 11 (6.2%)           | 5.66 ± 2.17               |         |
| Numbers of infectious complications |                  |                           | 0.214   |
| n = 1                    | 105 (59.3%)         | 6.91 ± 2.26               |         |
| n = 2                    | 65 (36.7%)          | 6.53 ± 2.05               |         |
| n = 3                    | 7 (4.0%)            | 5.86 ± 1.60               |         |

Table 4. Univariate and multivariate logistic regression analyses of infectious complications

| Variable   | Univariate analysis | P-value | Multivariate analysis | P-value |
|------------|---------------------|---------|-----------------------|---------|
|            | OR (95% CI)         |         | OR (95% CI)           |         |
| Age        | 1.033 (1.007–1.059) | 0.012   | 1.035 (1.008–1.063)   | 0.010   |
| ALB        | 0.951 (0.907–0.997) | 0.036   |                       |         |
| TBIL       | 1.003 (1.001–1.005) | 0.009   | 1.003 (1.001–1.005)   | 0.009   |
| PT         | 1.038 (1.002–1.075) | 0.038   |                       |         |
| Zn         | 0.838 (0.736–0.953) | 0.007   | 0.865 (0.755–0.990)   | 0.035   |

OR, odds ratio; CI, confidence interval; ALB, albumin; TBIL, total bilirubin; PT, prothrombin time; Zn, zinc.

Discussion

In this retrospective study, we found a high incidence (84.5%) of zinc deficiency in patients with HBV-related ACLF. The incidences of zinc deficiency in patients with non-cirrhosis, compensated cirrhosis, and decompensated cirrhosis were 75.9% (60/79), 94.0% (21/23), and 88.3% (159/180), respectively. Meanwhile, patients in the zinc deficiency group had a significantly higher MELD score than those in the subclinical zinc deficiency and normal zinc groups.

Interestingly, the most common infectious complications were peritonitis, pneumonia, and infectious diarrhea. It is worth noting that infectious complications were an independent predictor of the prognosis of patients with HBV-related ACLF. Further analyses indicated that zinc deficiency was an independent risk factor for infectious complications. It is well established that zinc plays an important role in immune responses [10]. Growing evidence suggests that immune cells are directly affected by zinc signals [12, 25]. Importantly, zinc regulates various functions of macrophages, including phagocytosis and the secretion of immune-mediating factors [11]. Moreover, zinc regulates phagocytosis, oxidative burst, and the production of pro-inflammatory cytokines by monocytes [26] and regulates natural killer cells, T cells, and B cells [27]. It is widely thought that achieving an optimal immune response to different stimuli and avoiding tissue and organ damage are a delicate balance determined by the regulation of zinc in extracellular and intracellular compartments [28]. These findings corroborate that zinc is essential for infection control, while zinc deficiency is involved in infectious diseases [13]. For instance, a study reported that alterations in zinc metabolism were more pronounced in septic patients than in non-infected critically ill patients [29]. In the context of sepsis, alteration of host zinc homeostasis is reportedly part of the host’s defense mechanism against pathogens [30]. Interestingly, Jolien et al. [31] found that zinc could inhibit lethal inflammatory shock by preventing the microbe-induced interferon signature in the intestinal epithelium. Herein, we found that HBV-related ACLF patients with a serum zinc level of <6.1 μmol/L were more likely to have infectious complications, which is in agreement with the theory that zinc deficiency is involved in infection [13].

Besides infectious complications, ascites, HE, GIB, and HRS were the main complications in HBV-related ACLF patients. Indeed, complications are closely related to the prognosis of patients with ACLF [32]. Our study suggested that the serum zinc level upon admission was significantly higher in patients without complications than in those with complications. It is worth noting that HE was a strong independent factor for evaluating the severity and predicting the outcome of patients with HBV-related ACLF [33]. HE was associated with low serum zinc levels in patients with acute liver failure [19]. A systematic review and meta-analysis showed that oral zinc supplementation was associated with significantly improved performance during the number connection test in cirrhotic patients with HE [34]. Zinc is safe; there is no evidence revealing recognized toxicity in humans [35]. Current evidence suggests that only acute and high levels of zinc exposure can induce respiratory and gastroenteric toxicity, which can be self-limited. Daily intake of >50 mg of zinc for >12 weeks can lead to copper deficiency, which presents as microcytic hypochromic anemia and neutropenia [36]. Adequate zinc supplementation may be a therapeutic option for controlling HE in patients with HBV-related ACLF.

The MELD score is a significant prognostic model for evaluating the severity and outcome of ACLF [20]. In our study, the highest serum zinc levels (7.57 ± 0.24 μmol/L), followed by those with 20% ≤ PTA < 30% (7.06 ± 0.21 μmol/L) and PTA < 20% (6.20 ± 0.21 μmol/L). Serum zinc levels in patients with PTA < 20% were significantly lower than those in patients with 20% ≤ PTA < 30% (P = 0.007) and those in patients with 30% ≤ PTA < 40% (P < 0.001).
MELD score was an independent factor associated with the prognosis of HBV-related ACLF patients. Patients in the zinc deficiency group had a higher MELD score than those in other groups. Moreover, the serum zinc level in HBV-related ACLF patients with coagulation disorders exhibited a gradual downward trend with the decrease in PTA. Given that coagulation disorders are the main manifestations in liver failure, parameters of coagulation function, such as PTA and PT-INR, are essential in staging ACLF in Chinese and APASL guidelines [4, 9]. In one Chinese guideline [4], ACLF patients are divided into three stages according to the PTA levels: early stage with 30% ≤ PTA < 40%, median stage with 20% ≤ PTA < 30%, and late stage with PTA < 20%. A low serum zinc level was significantly associated with poor coagulation function and a high MELD score. Accordingly, zinc is necessary for proper liver function, given that zinc deficiency has been implicated in the pathogenesis of liver diseases [37]. Tissue zinc depletion has been associated with impaired capacity to metabolize drugs in patients with chronic liver disease [38]. Zinc deficiency has been extensively studied in cirrhotic patients [17, 18, 24]. Ryuta et al. [39] found that hypozincemia was associated with hepatocarcinogenesis in HCV-related liver cirrhosis. Zinc supplementation has proved effective in Wilson’s disease treatment [40]. It has been shown that copper absorption is inhibited when high levels of metallothionein with a higher binding affinity for copper are induced in intestinal cells by zinc, while zinc can also induce metallothionein synthesis in the liver, leading to further neutralization of toxic copper [41]. Besides, zinc treatment can decrease the incidence of gastrointestinal disturbances, body weight loss, and mild anemia in patients with chronic hepatitis C [42]. As mentioned above, zinc supplementation has huge prospects for therapeutic application in controlling infection and HE, and improving the MELD score in patients with HBV-related ACLF. Screening for zinc deficiency and providing zinc supplementation may improve the outcomes of these patients. Notwithstanding that we provided compelling evidence that zinc deficiency is common in patients with HBV-related ACLF, it remains unclear whether zinc supplementation can improve the prognosis in patients with HBV-related ACLF, warranting further investigation.

The present study has several limitations and shortcomings. First, the included number of patients was relatively small since this was a single-center study. Moreover, the serum zinc level was not a direct parameter associated with prognosis in patients with HBV-related ACLF during univariate logistic regression analysis in this study, which may be attributed to the limited sample size. Indeed, future multicenter studies with larger sample sizes are required to increase the robustness of our findings. In addition, although we established that zinc deficiency is common in patients with HBV-related ACLF, we need to conduct further studies. Finally, although compensated cirrhosis is not a parameter associated with the prognosis in this study, the significantly greater number of patients with compensated cirrhosis in the zinc deficiency group may account for the poor outcomes and affect the reliability of our results to a certain extent.

In conclusion, zinc deficiency is common in patients with HBV-related ACLF. Zinc deficiency is significantly associated with infectious complications and MELD scores in patients with HBV-related ACLF.

Authors’ Contributions
All authors contributed to the concept and design. X.L. and W.X. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. X.W., L.W., and N.H. contributed to acquisition, analysis, and interpretation of the data. X.L. and W.X. drafted the manuscript. W.X. and L.P. obtained funding. W.X., L.P., and Y.M. took responsibility for the supervision of the study and the revision of the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of Interest
The authors declare that there is no conflict of interests in this study.

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