Prognostic Relevance of Anemia In Hospitalized Patients With Cirrhosis:A Multicenter Cohort Study

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Abstract

Background and Aims: Anemia is common in cirrhosis and closely related with adverse outcome in cirrhotic patients, but their clinical features and the effect of variations in hemoglobin levels on mortality or time of death have not yet fully elucidated. As we performed this prospective, multi-center, cohort study to further determine these points.

Methods: We retrospectively collected data from the Chinese AcuTe on CHronic LIver FailurE (CATCH-LIFE) study, a prospective multi-center cohort study of patients with chronic liver disease and acute exacerbation conducted by the Chinese Chronic Liver Failure (CLIF) Consortium, which is composed of 15 tertiary hospitals in hepatitis-B-virus high endemic area. Patients were consisted of two prospective multi-center cohorts from the CLIF Consortium between January 2015 to December 2016 and July 2018 to January 2019.We conducted data analysis on prevalence of anemia and clinical characteristics among these patients and further determine the relationship between anemia and prognosis.

Results: Among 1979 hospitalized patients with cirrhosis,1389(70.2%) were combine with anemia, a patient has an 6.8% lower risk of death on 90-day mortality and 5.7% lower risk of death on 1-year mortality with a 10g/L decrease in hemoglobin at any level throughout the entire range. Patients with severe anemia have significant higher risk [HR=1.649(1.100,2.473), p=0.016] of 90-day mortality and [HR=1.610(1.159,2.238), p=0.005] of 1-year mortality comparedto those without anemia.

Conclusions: Anemia is common in cirrhosis and strongly associated with increased mortality after acute decompensation (AD)/acute liver injury (ALI). Severe anemia is an independent risk factor for poor 90-day and 1-year prognosis in cirrhosis with AD/ALI.

Introduction

Anemia, defined by the WHO as a reduction in the concentration of hemoglobin (< 120 g/L for women and < 130 g/L for men) [1], is common in patients with cirrhosis. The underlying mechanism is multifaceted. For example, overt or occult gastrointestinal bleeding from either esophageal or gastric varices and portal PHG play an important role [2], and the cirrhosis-associated chronic inflammatory state resulting from intestinal microbial translocation leads to chronic inflammatory anemia in cirrhosis [3]. Besides, low hepcidin concentration, folate deficiency, malnutrition, hypersplenism, derangement of the hematopoietic niche and loss of HSCs, alcohol and bilirubin toxicity and renal insufficiency could also be a significant cause of anemia in advanced cirrhosis [4–7].

Several studies have shown the clinical significance of anemia in patients with cirrhosis. A retrospective cohort study reported anemia is a strong predictor of hospitalization due to hepatic decompensation in outpatients with liver cirrhosis [8]. And it is reported that the incidence of ACLF was significantly higher in patients with anemia than those without anemia [9], as low hemoglobin concentrations were independently associated with cerebral hypoxia in patients with decompensated cirrhosis and could be a trigger for ACLF. Furthermore, distinct mechanisms of anemia have also been demonstrated to be associated with poor prognosis in cirrhosis, for example, macrocytic anemia was found to be associated with the severity of liver damage and might be a predictor for short-term mortality in patients with HBV-related decompensated cirrhosis [10] and increased mortality was observed among patients with spur cell anemia after splenectomy or alcoholism because of progressive lipoprotein impairment [11]. IDA, which is common in cirrhosis was also found significantly associated with an increased risk of mortality in cirrhosis [12].

Despite of these, mostly previous studies were retrospective and single-center which included only a relatively limited size of cases, the effect of variations in hemoglobin levels on mortality and time of death have not yet fully elucidated, thus a large prospective cohort study is required to further determine the association between hemoglobin levels and adverse outcome in patients with cirrhosis. In the present study, we performed this prospective, multicenter cohort study to

determine the prevalence and severity of anemia among cirrhotic patients with AD or ALI and further explored the association between anemia and mortality at 28-day, 90-day and 1-year follow up among these patients.

Patients And Methods

Study design and population

We retrospectively used data from the CATCH-LIFE study (NCT02457637, NCT03641872), a prospective multicenter cohort study of patients with chronic liver disease and acute exacerbation conducted by the CLIF Consortium, which is composed of 15 tertiary hospitals in HBV high endemic area. Patients were consisted of two prospective multicenter cohorts from the CLIF Consortium between January 2015 to December 2016[13] and July 2018 to January 2019.

We collected patients with cirrhosis who were admitted to hospital due to acute AD or ALI. The exclusion criteria were: Acute gastrointestinal bleeding in two weeks before admission. Hepatocellular carcinoma or other liver malignancies before or during admission; Extrahepatic malignancies or severe chronic extrahepatic disease; Age younger than 18 or older than 80 years and pregnancy; Receiving immunosuppressive agents for non-hepatic diseases. The study adheres to the Declaration of Helsinki and was approved by The Renji Hospital Ethics Committee of Shanghai Jiaotong University School of Medicine, and all the study patients gave their informed written consent. All authors had access to the study data and had reviewed and approved the final manuscript.

Participants Follow-up

Follow-up data were collected from all the patients during hospitalization within 28 days and obtained regularly from outpatient follow-up or telephone contact after discharge. Patients were considered to be off study if they died, were lost to follow-up, developed malignancies, received LT, or withdrew informed consent. The primary endpoint was mortality at 28-day, 90-day and 1-year. On admission, the patients were recorded for demographic information and medical history. During hospitalization, laboratory parameters, radiological findings, complications and therapies were recorded at 1, 4, 7, 14, 21, and 28 days (or the last day if the patient was hospitalized for less than 28 days) as well as 24 hours prior to death or LT (if the patient died or had LT), models for end-stage liver disease scores, sepsis and organ failure were evaluate based on available data. After hospital discharge, all patients underwent follow-up regularly from outpatient review or telephone monthly, clinical outcome as death, LT, and developing into malignancies were recorded, if patients died, then the time of death and the main cause of death were noted. Up to 1 year, approximately 3% of patients were unable to be contact (loss to follow) by phone, for reasons including not answering the phone, wrong number, refusal to answer questions, a number not in service, and other reasons. All patients received routine treatment according to relevant guidelines during hospitalization, and after discharge, healthy life habits, long-term antiviral therapy, alcohol intake restrictions and other symptomatic therapy are monitored during follow-up.

Definition

Anemia was defined as a reduction in the concentration of hemoglobin (< 120 g/L for women and < 130 g/L for men) by WHO as previous described[1], and was divided into different degrees, with mild anemia ranging from 110g/L to 120g/L in non-pregnant women(15 years of age and above) and 110g/L to 130g/L in men(15 years of age and above), moderate anemia ranging from 80g/L to 109g/L and severe anemia under 80g/L. Cirrhosis was diagnosed based on CT/MRI scan, laboratory tests, clinical symptoms and history of liver disease. Acute decompensation was defined as the acute development of gastrointestinal hemorrhage, hepatic encephalopathy, overt ascites, bacterial infection, or jaundice (total bilirubin > 5 mg/dl) or any combination of these within one month before enrollment [14, 15] and acute liver injury was defined as alanine aminotransferase or aspartate aminotransferase levels > 3 times the upper limit of normal or a total bilirubin level > 2 times the upper limit of normal within 1 week.

Statistical analysis

All statistical analyses were conducted using R (version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria). Continuous variables between two groups were compared with Student t tests or the Mann–Whitney U test and presented as the medians with interquartile ranges. Categorical variables were compared with the chi-squared or the Fisher exact tests and represented as counts and percentages. For multiple groups, Fisher exact test or a $\chi 2$ test was used for group comparisons for categorical variables, for continuous variables, comparison was performed by one-way ANOVA for data with normal distribution and for data with nonnormal distribution, Kruskal-Wallis one-way ANOVA on ranks was used. Post-hoc Tukey pairwise comparisons were used for post hoc pairwise comparisons. Multiple imputation methods were used to deal with missing values. Univariate Cox analysis and multivariate analysis was performed using the Cox Proportional hazards model in Stratified Analyses, and was also used to determine the relationship between the severity of anemia and mortality. Multinomial logistical regression analysis was performed to identify factors associated with multiple outcomes (within 28-day death, post-28-day death, and survival). GAM were used to evaluate the curvilinear association between anemia and LT-free mortality. The 90-day and 1-year LT-free survival curves were plotted and comparisons between groups were performed by the Kaplan–Meier method. P-values less than 0.05 ($P < 0.05$) were considered statistically significant.

Results

Baseline Characteristics of the overall Study Population

Screening steps of the study population was shown in **Figure.1**. Notably, we firstly excluded 1144 patients without cirrhosis and 577 cirrhotic patients with acute gastrointestinal bleeding at admission. Finally, 1979 patients with cirrhosis who were admitted to hospital due to AD or ALI were included in our study.

Clinical characteristics of the cohort and subgroups of patients with/without anemia was shown in **Table.1**. Among 1979 patients, the median age is 51(IQR 35–67) years, with 1437(72.6%) patients were male. The median hemoglobin was 115g/L (IQR 73–147), and the overall mortality at 28-day, 90-day and 1-year were 10.4%, 18.9% and 27.4% respectively. As compared with patients without anemia, those with anemia often combine with higher 28-day,90-day and 1-year mortality, higher prognostic scores, higher levels of laboratory parameters such as serum bilirubin, international normalized ratio, creatinine, NLR and so on, and lower levels of laboratory parameters such as albumin, platelet count and serum sodium and so on.

Baseline characteristics of patients with varying degrees of anemia was shown in **Table.2**. Among 1389 patients with anemia, 599(41.3%) patients were combined with mild anemia, 595(15.8%) with moderate anemia and 195(2.4%) with severe anemia. As expected, with exacerbating of anemia, there was a step-wise increase of 90-day and 1-year mortality and laboratory parameters such as serum bilirubin,international normalized ratio, creatinine, NLR and so on, and a step-wise of decline of laboratory parameters such as albumin, platelet count and serum sodium and so on.

Anemia and Mortality

Effect of anemia on mortality: stratified analyze

As a variety of demographic and clinical parameters significantly differed among patients with varying degree of anemia and those without, a stratified analysis was performed to analyze the variables that impact on the association between hemoglobin levels and LT-free 90-day and 1-year mortality. As shown in **Figure.3**, if unadjusted, hemoglobin level was closely related to increased mortality at 90-day follow-up [HR = 0.995(0.991,0.999)]. Adjusted by gender and WBC level remained to be significantly associated to increased mortality. However, the significant association between hemoglobin level and mortality was altered in at least one of two subgroups stratified by age, cirrhosis etiology, TB, Cr, INR, ALT, AST,

ALB, PLT, presence of infection, ascites or HE, suggesting a potential interactive effect between hemoglobin level and these variables.

Similarly, as shown in **Figure.4**, if unadjusted, hemoglobin level was closely related to 1-year increased mortality [HR = 0.992(0.988,0.995)]. Adjusted by age, PLT count, level of serum bilirubin and presence of infection, decreased hemoglobin level remained to be significantly associated to increased mortality. However, the significant association between hemoglobin level and mortality was altered in one of two subgroups stratified by gender, cirrhosis etiology, ALT, AST, WBC, presence of ascites or HE, suggesting a potential interactive effect between hemoglobin level and these variables.

Effect of HB level on mortality

Effect of HB level on 28-day,90-day and 1-year mortality

We performed univariate Cox analysis and multivariate Cox analysis to determine the relationship between hemoglobin levels and 28-day, 90-day and 1-year mortality. As is shown in **Table.3**, insufficient evidence suggested that there exists significant relationship between HB level and 28-day mortality adjusted by different models, moreover, presence of any degree of anemia was also not significantly associated with increased 28-day mortality adjust to different models.

As shown in **Table.3**,hemoglobin level was proved to be independently associated with 90-day mortality adjusted by different models with an hazard ratio of 0.993 (95% CI, 0.988–0.998, p = 0.006) in the fully adjusted Model(Adjusted for age, gender, etiology, ascites, HE, infection, TB, INR, CR, ALB,ALT,AST,WBC, PLT, Na) and when divided into various degrees of anemia, severe anemia was proved to be associated with 90-day mortality by different models, with an hazard ratio of 1.649 (95% CI, 1.100-2.473 p = 0.016) in the fully adjusted Model (eg, patients with severe anemia got 1.649-fold risk of death compared with patients without severe anemia on 90-day mortality).

Likewise, hemoglobin level was also proved to be independently associated with 1-year mortality adjusted by different models,with an hazard ratio of 0.994 (95% CI, 0.990–0.998, p = 0.006) in the fully adjusted Model and when divided into various degrees of anemia, we identified that severe anemia is an independent risk factor for increased 1-year mortality, with an hazard ratio of 1.610 (95% CI, 1.159–2.238 p = 0.005) in the fully adjusted Model (eg, patients with severe anemia got 1.610-fold risk of death compared with patients without anemia on 1-year mortality).

As shown in **Figure.4**, generalized additive model was performed to evaluate the curvilinear association between anemia and 90-day mortality, in which the mortality increased significantly with the reduction of the HB level. In all-adjusted model, the effect of HB level on death was nearly perfectly linear, with an adjusted odds ratio of 0.932 (95% CI, 0.886-980,p = 0.006) associated with a 10g/L decrease in HB at any level in our study population (eg, a patient with a HB of 80g/L has an 6.8% lower adjusted hazard of death than one with a HB of 90g/L, and so on throughout the entire range). Similarly, generalized additive model was also performed to evaluate the curvilinear association between anemia and 1-year mortality in **Figure.4**, in which the mortality increased significantly with the reduction of the HB level. In all-adjusted model, the effect of HB level on death was nearly perfectly linear, with an adjusted odds ratio of 0.943 (95% CI, 0.905–0.983 p = 0.006) associated with a 10g/L decrease in HB at any level in our study population (eg, a patient with a HB of 80g/L has an 5.7% lower adjusted odds of death than one with a HB of 90g/L, and so on throughout the entire range).

Effect of severe anemia on early/delayed mortality

Survival curves were made between patients with/without severe anemia to describe the relationship between HB and LT-free mortality, in which statistical significance was shown that patients with severe anemia had higher 90-day and 1-year mortality(**Figure.5**), moreover, based on the above finding that anemia is not a risk factor for early death(within 28-day mortality) in our study population, we then performed a multinominal logistic regression analysis to determine significant risk factors for increased mortality within 28-day(early death)/28–365 day(delayed death). As shown in **Table.4**, older age,

high levels of ALT, TB, WBC, INR, CR, lower levels of Na, ALB and presence of HE were predictors of 28-day mortality, while factors only significant associated with 28–365 day mortality were male, presence of ascites and severe anemia.

Discussion

We performed this large multi-center prospective cohort study to describe the prevalence of anemia among cirrhotic patients with AD/ALI and then investigated the impact of anemia on patient outcome. We found hemoglobin levels are independently associated with increased 90-day and 1-year mortality, as a patient has an 6.8% lower adjusted hazard risk of death on 90-day mortality and 5.7% lower adjusted hazard risk of death on 1-year mortality with a 10g/L decrease in hemoglobin at any level throughout the entire range. With regards to severity of anemia, severe anemia is an independent risk factor for increased 90-day and 1-year mortality in cirrhotic patients with AD/ALI.

Anemia is common in patients with cirrhosis and previous studies have demonstrated the remarkable decrease of hemoglobin concentration with an incidence ranging from 21%-84% among patients with varying severity of cirrhosis[2,10□16□17]. The results is in line with our study, as we reported an incidence up to 70.2%, with 9.9% combine with severe anemia. The potential causes of anemia include portal hypertension, chronic inflammatory condition, bone marrow suppression, the presence of malnutrition and imbalances of iron homeostasis are more common in cirrhosis as previously described [2–7]. Remarkably, patients with alcohol-related cirrhosis have higher incidence of anemia compared with HBV-related cirrhosis, with an incidence of anemia 80.7–65.8% in our study population. Possible explanations are as following: firstly, bone marrow toxicity of alcohol could be an important reason for the development of anemia[15], secondly, hemolytic anemia were shown in patients with alcohol-related cirrhosis as previous described through altering the structure and metabolic pathways of red-blood cell membrane, moreover, malnutrition is common in patients with alcohol-related cirrhosis as chronic alcohol consumption may lead to micronutrient deficiencies[18], in addition, spur cell anemia caused by alcohol-related cirrhosis have also been reported by previous studies[19, 20].

A strong linkage between anemia and adverse outcome was also found in patients with cirrhosis. Firstly, anemia was significantly associated with illness severity, as patients with anemia often combine with higher prognostic scores, higher levels of laboratory parameters such as serum bilirubin, international normalized ratio, creatinine, NLR and lower levels of laboratory parameters such as albumin, platelet count and serum sodium which could indicate the severity of the disease as previous described, the result is in line with a previous study which have demonstrated the closely relationship between anemia and ACLF[9]. Moreover, our study confirmed that HB level was an independent risk factor for increased mortality among patients with cirrhosis. Specifically, severe anemia is associated with increased 90-day and 1-year mortality, rather than 28-day mortality, among patients with cirrhosis is the central viewpoint of this study, which may be related to the following factors.

First, anemia may accelerate the progression of liver cirrhosis through affecting the hemodynamic status. It has been shown that low hemoglobin level results in hemodilution and the reduction of the blood viscosity, aggravates tissue hypoxia and increases the cardiac output, and finally worsen the hyperdynamic circulation and contributes to the development of portal hypertension [21].

Second, anemia may increase the risk of decompensation events. For example, in addition to exacerbating portal hypertension, reduced level of hemoglobin leads to decline of NO scavenging and subsequent guanylyl cyclase activation which impairs platelet aggregation[22] and increases risk of bleeding. Also, anemia is associated with an increased risk of HE, which is an independent factor for adverse outcome in cirrhosis. In our study,the incidence of HE in patients with moderate to severe anemia was 16.5% compared with 9.7% among those without anemia□in line with a prospective cohort study that reported a relationship between anemia and HE in ambulatory cirrhotic patients without recent overt gastrointestinal bleeding on baseline[23]. It is shown that anemia is associated with hyperammonemia due to occult gastrointestinal blood loss in cirrhosis [24]. Further, patients with anemia have increased risk of infection□with an

incidence up to 31.7% compared with 26.5% in patients without anemia in our study. Previous studies have shown that low hepcidin level impose increased vulnerability to bacterial infection in patients with cirrhosis and IDA [25].

Besides, anemia may imply the presence of derangement of the hematopoietic niche and loss of HSCs which is correlated with severity of cirrhosis and cause the hematological and immunological dysfunctions in patients with advanced cirrhosis [5]. These mechanisms suggest that anemia is a non-ignorable risk factor in the temporal course of disease progression in patients with cirrhosis and could result in an increased long-term mortality.

The strength of study is the high-quality data based on a multi-center, prospective, national cohort. However, we admit several limitations in the study. First, the diagnosis of anemia is based on hemoglobin level obtained at admission and we did not include those developed into anemia during hospitalization. Second, the possible causes of anemia were not further explored based on our limited available data. Finally, we did not assess the recurrence of decompensated events after hospital discharge during 1-year follow-up, thus the effect of anemia on risk of recurrent de-compensation remain to be determined.

Conclusion

In conclusion, anemia is common in various types of cirrhosis and were closely correlated with the severity of liver disease. Hemoglobin levels are strongly associated with increased 90-day and 1-year mortality, and Severe anemia is an independent risk factor for poor prognosis among cirrhotic patients with AD/ALI. Potential clinical benefit of correcting anemia such as the use of erythropoietin is warrant to be assessed.

Declarations

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Conflict of interest

The authors disclose no conflicts.

Ethical approval

Data was from the CATCH-LIFE study (NCT02457637, NCT03641872).The study adheres to the Declaration of Helsinki and was approved by The Renji Hospital Ethics Committee of Shanghai Jiaotong University School of Medicine

Consent to participate

All the study patients gave their informed written consent.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and material

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

Haotang Ren , Liang Qiao, Baoyan Xu, Yixin Hou, Yan Xiong, JunjinChen, Beiling Li, Sen Luo, Na Gao and Weituo Zhang collected and analysed the data; Yu Shi, Hai Li, Guohong Deng, Xianbo Wang, Xin Zheng,Yan Huang, Jinjun Chen, Zhongji Meng, Yanhang Gao, Zhiping Qian,Feng Liu, Xiaobo Lu, Jia Shang , Hai Li and Shaoyang Wang designed the research study; Haotang Ren wrote the paper and Yu Shi, Hai Li, Guohong Deng, Xianbo Wang, Xin Zheng,Yan Huang, Jinjun Chen, Zhongji Meng, Yanhang Gao, Zhiping Qian,Feng Liu, Xiaobo Lu critically reviewed the manuscript. All authors approved the final version of the manuscript.

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Abbreviations

WHO World Health Organization

PHG portal hypertensive gastropathy

HSCs hematopoietic stem cells

ACLF acute-on-chronic liver failure

HBV hepatitis B virus

IDA Iron deficiency anemia

AD acute decompensation

ALI acute liver injury

CATCH-LIFE Chinese AcuTe on CHronic LIver FailurE

CLIF Chinese Chronic Liver Failure

LT liver transplantation

GAM Generalized additive model

HB hemoglobin

HE hepatic encephalopathy

ALT alanine aminotransferase;

AST aspartate aminotransferase;

TB total bilirubin;

WBC white blood cell count;

NLR neutrophil-to-lymphocyte ratio;

ALB albumin

MELD Model for End Stage Liver Disease.

INR International normalized ratio

PLT platelet count

Tables

Table.1 Baseline characteristics of study population

Variables	Total (n=1979)	No anemia (n=590)	Anemia (n=1389)	P value
Demographic				
Age	51(16)	49(15)	52(15)	<0.001
Male	1437(72.6)	458(77.6)	979(70.5)	<0.001
Etiology				
HBV	1188(60.0)	406(68.8)	782(56.3)	<0.001
Alcohol	212(10.7)	41(6.9)	171(12.3)	<0.001
HBV plus alcohol	190(9.6)	64(10.8)	126(9.1)	<0.001
Others	389(19.7)	79(13.4)	310(22.4)	<0.001
AD				
Infection	585(29.6)	145(24.6)	440(31.7)	<0.001
Ascites	1316(66.5)	310(52.5)	1006(72.4)	<0.001
Jaundice	1025(51.8)	321(54.4)	704(50.7)	<0.001
HE	220(11.1)	54(9.2)	166(12.0)	<0.001
Laboratory parameters				
ALT (IU/L)	67(182)	218(516)	52(90)	<0.001
AST(IU/L)	94(158)	179(328)	78(110)	<0.001
ALB (g/dL)	30.3(7.5)	32.6(7.1)	29.1(7.4)	<0.001
TB (μmol/L)	5.4(14.0)	7.1(16.3)	5.2(13.0)	0.041
HB(g/L)	115(32)	137(15)	106(27)	<0.001
INR	1.51(0.64)	1.45(0.60)	1.54(0.67)	0.043
WBC count (10^9/L)	4.9(3.5)	5.4(3.2)	4.6(3.6)	0.001
Cr (μmol/L)	0.78(0.34)	0.76(0.28)	0.79(0.38)	<0.001
PLT (10^9/L)	78(67)	95(69)	72(62)	<0.001
Serum sodium (mmol/L)	138.0(6.0)	138.9(5.0)	137.3(6.0)	<0.001
NLR	2.7(3.0)	2.5(2.8)	2.7(3.1)	<0.001
Scores				
MELD	19.7(16.8)	18.6(24.7)	20.6(17.2)	<0.001
MELD_Na	16.1(21.7)	12.5(18.0)	17.7(22.2)	<0.001
iMELD	38.9(19.0)	36.0(16.5)	40.6(18.7)	<0.001
Mortality(%)				
28-day	205(10.4)	60(10.2)	145(10.4)	0.984
90-day	374(18.9)	102(17.3)	272(19.6)	0.491
1-year	538(27.2)	129(21.9)	409(29.4)	0.002

Data are expressed as mean ± SD, median (interquartile range), or number(percent).

Table.2 Baseline characteristics of patients with varying degrees of anemia

Variables	Mild anemia (n=599)	Moderate anemia (n=595)	Sever anemia (n=195)	P value
Demographic				
Age	51(15)	53(16)	52(15)	<0.001
Male	492(82.1)	372(62.5)	115(59.0)	<0.001
Etiology				
HBV	377(62.9)	320(53.8)	85(43.6)	<0.001
Alcohol	60(10.0)	69(11.6)	42(21.5)	<0.001
HBV plus alcohol	75(12.5)	40(6.7)	11(5.6)	<0.001
Others	87(14.5)	166(27.9)	57(29.2)	<0.001
AD				
Infection	170(28.4)	206(34.6)	64(32.8)	0.064
Ascites	402(67.1)	444(74.6)	160(82.1)	<0.001
Jaundice	312(52.1)	310(52.1)	82(42.1)	0.035
HE	62(10.4)	68(11.4)	36(18.5)	0.009
Laboratory parameters				
ALT (IU/L)	81(166)	45(62)	25(26)	<0.001
AST(IU/L)	103(157)	76(97)	43(49)	<0.001
ALB (g/dL)	29.7(6.9)	28.5(7.6)	28.7(7.6)	0.001
TB (μmol/L)	5.5(15.2)	5.5(11.1)	3.4(9.8)	0.001
HB(g/L)	118(10)	98(14)	69(14)	<0.001
INR	1.54(0.67)	1.54(0.66)	1.53(0.71)	0.879
WBC count (10^9 /L)	5.0(3.4)	4.5(3.6)	4.0(4.0)	<0.001
Cr (μmol/L)	0.79(0.32)	0.78(0.37)	0.79(0.50)	0.060
PLT (10^9 /L)	75(62)	71(59)	63(42)	0.109
Serum sodium (mmol/L)	137.8(6.0)	137.2(6.0)	136.0(8.0)	0.002
NLR	2.5(3.1)	2.7(3.2)	3.5(3.1)	<0.001
Scores				
MELD	19.9(16.0)	21.2(17.7)	18.6(21.5)	0.092
MELD_Na	16.4(19.6)	18.7(23.1)	18.0(26.2)	0.020
iMELD	38.9(17.1)	41.9(20.2)	39.7(23.5)	0.005
Mortality (%)				
28-day	54(9.0)	70(11.8)	21(10.8)	0.295
90-day	89(14.9)	136(22.9)	47(24.1)	0.001

1-year	136(22.7)	198(33.3)	75(38.5)	<0.001

Data are expressed as mean±SD, median (interquartile range), or number(percent).

Table.3 Univariate nd multivariate Cox analysis of the realtionship between anemia and 28-day,90-day and 1-year mortality

Variable	Num of 28-day mortality (percentage)	HR, 95% CI, P-value	HR, 95% CI, P-value	HR, 95% CI, P-value	HR, 95% CI, P-value
		Model I	Model II	Model Ⅲ	Model Ⅳ
HB	205(10.4)	0.999(0.993,1.004), p=0.649	0.996(0.990,1.002), p=0.189	0.997(0.990,1.003), p=0.270	0.994(0.987,1.001), p=0.101
Severity of anemia					
No anemia	60(10.2)	1.0	1.0	1.0	1.0
Mild anemia	54(9.0)	0.875(0.606,1,263), p=0.475	0.829(0.573,1.198), p=0.318	0.811(0.555,1.185), p=0.279	0.884(0.593,1.318), p=0.545
Moderate anemia	70(11.8)	1.150(0.814,1.623), p=0.427	1.197(0.841,1.703), p=0.319	1.075(0.744,1.555), p=0.700	1.184(0.788,1.781), p=0.416
Sever anemia	21(10.8)	1.069(0.650,1.757), p=0.793	1.261(0.758,2.096), p=0.372	1.294(0.765.2.189), p=0.337	1.443(0.808,2.578), p=0.215
Variable	Num of 90-day mortality (percentage)	HR, 95% CI, P-value	HR, 95% CI, P-value	HR, 95% CI, P-value	HR, 95% CI, P-value
Severity of anemia		Model I	Model II	Model Ⅲ	Model Ⅳ
HB	374(18.9)	**0.995(0.991,0.999), p=0.011**	**0.993(0.989,0.997), p=001**	**0.995(0.990,0.999), p=0.023**	**0.993(0.988,0.998), p=0.006**
Severity of anemia					
No anemia	102(17.3)	1.0	1.0	1.0	1.0
Mild anemia	89(14.9)	0.842(0.634,1.119), p=0.236	0.812(0.610,1.080), p=0.152	0.751(0.561,1.004), p=0.053	0.789(0.581,1.072), p=0.130
Moderate anemia	136(22.9)	1.339(1.035,1.730), p=0.026	1.374(1.057,1.787), p=0.018	1.152(0.877,1.512), p=0.310	1.230(0.909,1.664), p=0.180
Sever anemia	47(24.1)	**1.419(1.004,2.005), p=0.047**	**1.570(1.101,2.239), p=0.013**	**1.511(1.047,2.182), p=0.028**	**1.649(1.100,2.473), p=0.016**
Variable	Num of 1-year mortality (percentage)	HR, 95% CI, P-value	HR, 95% CI, P-value	HR, 95% CI, P-value	HR, 95% CI, P-value
Severity of anemia		Model I	Model II	Model Ⅲ	Model Ⅳ
HB	538(27.2)	**0.992(0.988,0.995), p<0.001**	**0.992(0.988,0.995), p<0.001**	**0.994(0.990,0.998), p=0.002**	**0.994(0.990,0.998), p=0.006**
Severity of					

anemia					
No anemia	**129(21.9)**	1.0	1.0	1.0	1.0
Mild anemia	**136(22.7)**	1.023(0.804,1.302), p=0.853	0.978(0.768,1.245), p=0.856	0.893(0.699,1.140), p=0.362	.0.868(0.671,1.123), p=0.282
Moderate anemia	**198(33.3)**	1.587(1.272,1.982), p<0.001	1.514(1.207,1.899), p<0.001	1.222(0.967,1.544), p=0.093	1.181(0.914,1.527), p=0.204
Sever anemia	**75(38.5)**	**1.863(1.401,2.476), p<0.001**	**1.840(1.374,2.465), p<0.001**	**1.675(1.238,2.265), p=0.001**	**1.610(1.159,2.238), p=0.005**

Model I Un-adjusted;

Model II Adjusted for age gender etiology;

Model Adjusted for age gender etiology HE ascites infection, TB INR Cr

Model (full-adjusted) Adjusted for age, gender, etiology, ascites, HE, infection, TB, INR, CR, ALB,ALT,AST,WBC, PLT, Na

Table.4 Risk factors associated with early/delayed death by multivariate multinomial logistic regression analysis

Variable	Early death (within 28 days) OR, 95% CI	P-value	Delayed death (28 days-1 year) OR,95% CI	P-value
Severe anemia	1.544(0.844,2.861)	0.157	**1.789(1.189,2.692)**	**0.005**
Age	**1.060(1.040,1.079)**	**<0.001**	**1.034(1.020,1.048)**	**<0.001**
Male	0.721(0.460,1.131)	0,155	**0.611(0.448,0.832)**	**0.002**
AST	1.000(0.999,1.001)	0.761	1.001(1.000,1.001)	0.202
ALB	**0.942(0.907.0.978)**	**0.002**	**0.960(0.935,0.985)**	**0.002**
ALT	**1.001(1.000,1.001)**	**0.011**	**0.999(0.998,1.000)**	**0.033**
TB	**1.097(1.076,1.118)**	**<0.001**	**1.067(1.050,1.084)**	**<0.001**
INR	**2.422(1.887,3.110)**	**<0.001**	**1.776(1.405,2.245)**	**<0.001**
PLT	0.997(0.994,1.001)	0.127	1.000(0.998,1.001)	0.673
CR	**1.359(1.098,1.683)**	**0.005**	1.207(0.992,1.469)	0.060
WBC	**10.84(1.036,1.135)**	**<0.001**	1.024(0.984,1.065)	0.243
Na	**0.925(0.895,0.956)**	**<0.001**	**0.944(0.920,0.969)**	**<0.001**
HBV-related	1.554(0.844,2.861)	0.157	0.842(0.634,1.117)	0.233
Presence of HE	**2.957(1.807,4.839)**	**<0.001**	**1.949(1.283,2.959)**	**0.002**
Presence of Ascites	1.496(0.973,2.300)	0.066	**1.607(1.160,2.226)**	**0.004**
Presence of Infection	0.983(0.668,1.448)	0.933	1.254(0.936,1.680)	0.130

Statistical ananlysis was performed using multinominal logistic regression model to identify risk factors associated with multiple outcomes(survival, early/delayed death)

Figures

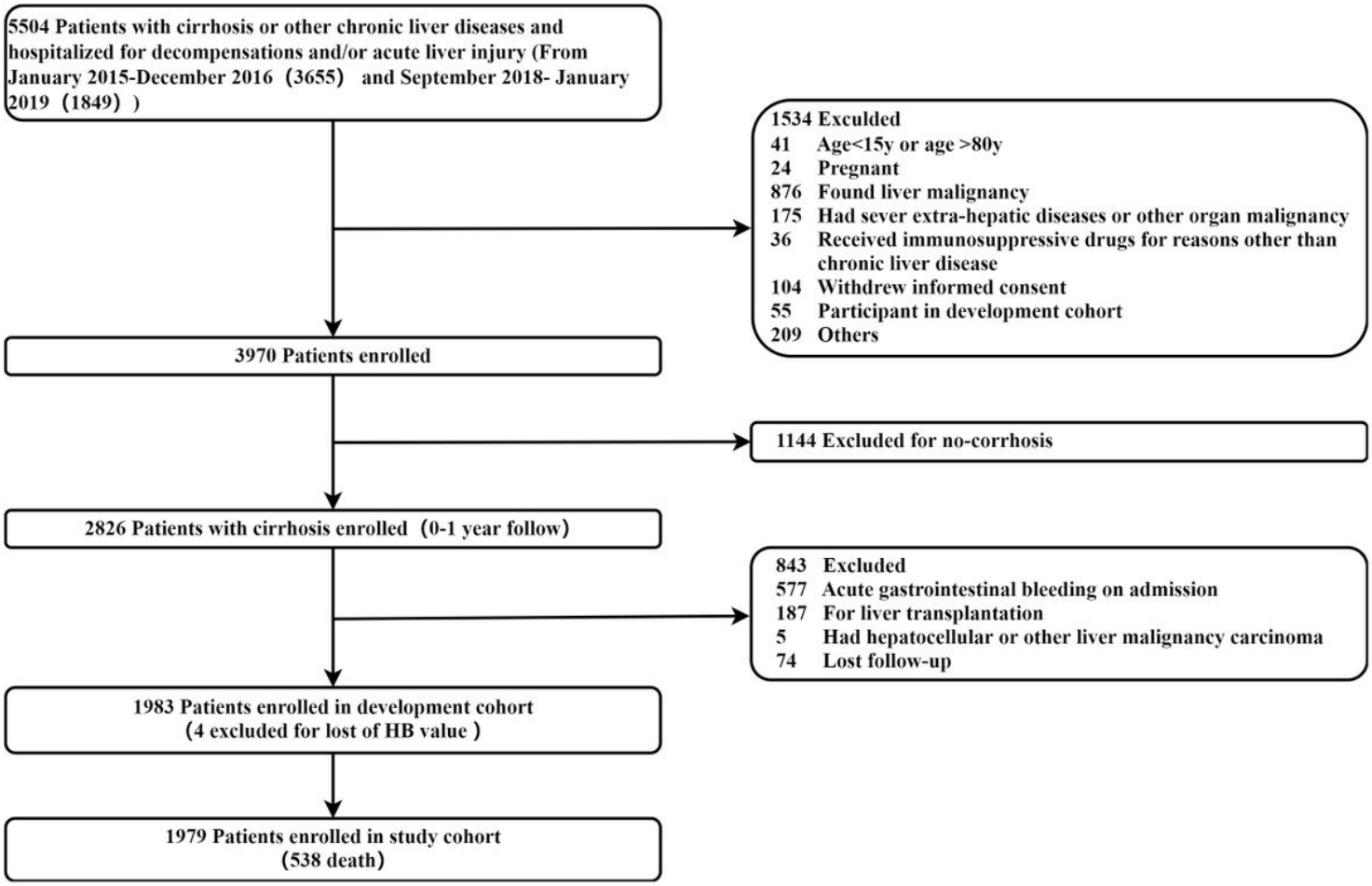

Figure 1

Flow chart of study cohort

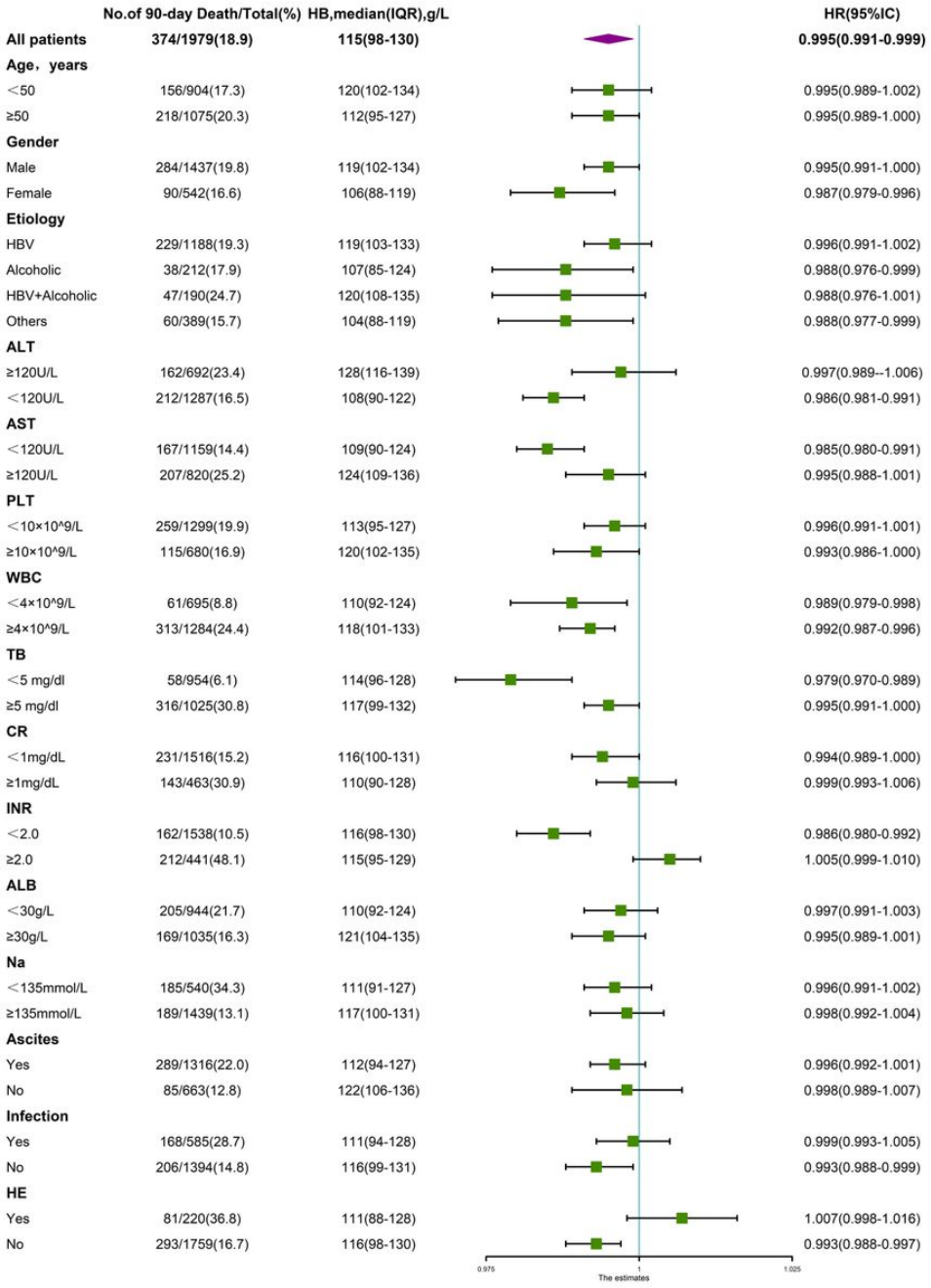

Figure 2

Stratified analyses of risk of 90-day death according to hemoglobin levels. The unadjusted hazard ratio of death per unit increment in standard deviation of hemoglobin is plotted for the entire cohort and according to strata of baseline covariates. Subgroups were stratified by age, gender, etiology, ALT, AST, PLT, WBC, TB, CR, INR, ALB, Na, precence of ascites, infection and HE.

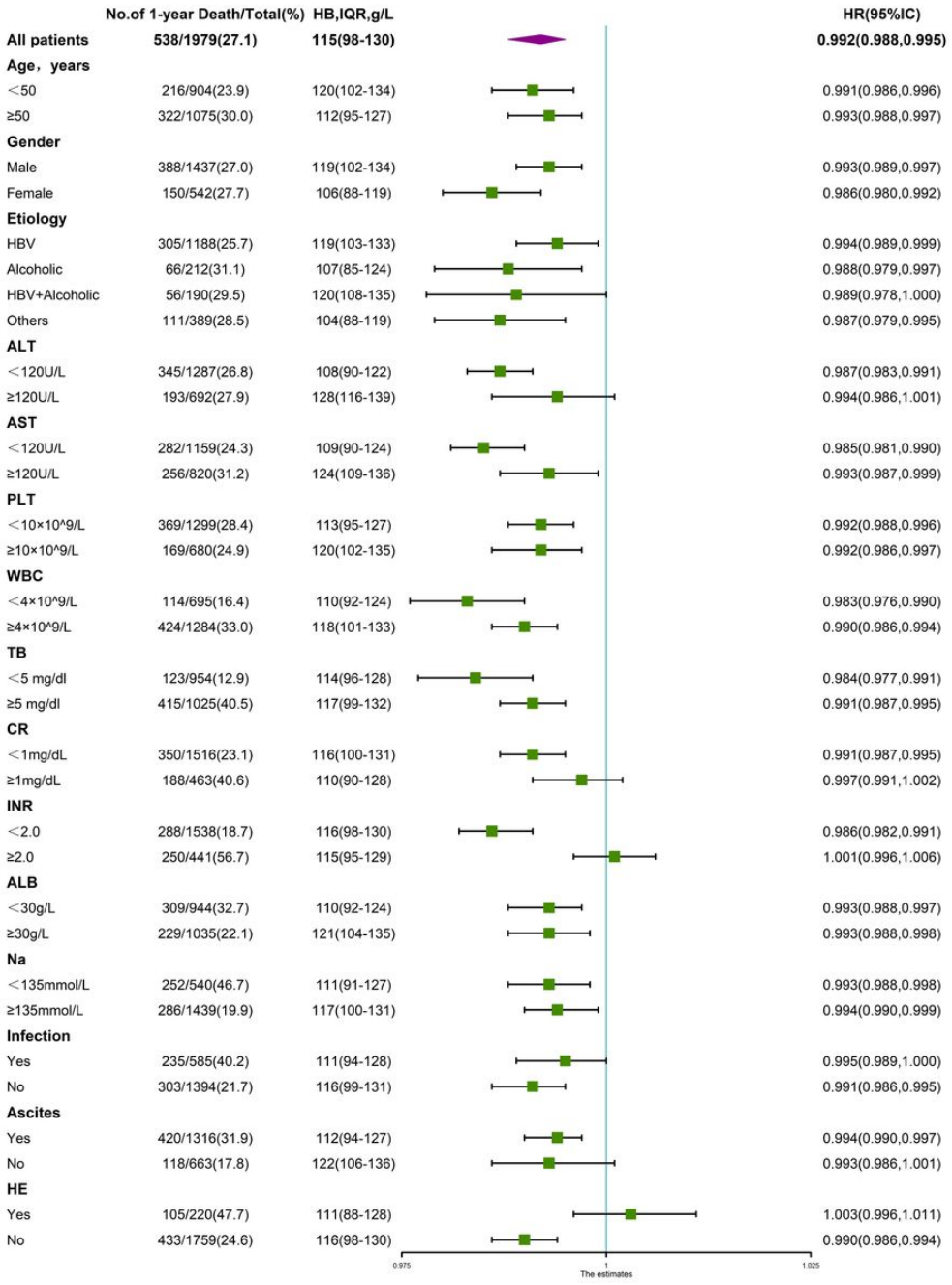

Figure 3

Stratified analyses of risk of 1-year death according to hemoglobin levels. The unadjusted hazard ratio of death per unit increment in standard deviation of hemoglobin is plotted for the entire cohort and according to strata of baseline covariates. Subgroups were stratified by age, gender, etiology, ALT, AST, PLT, WBC, TB, CR, INR, ALB, Na, presence of ascites, infection and HE.

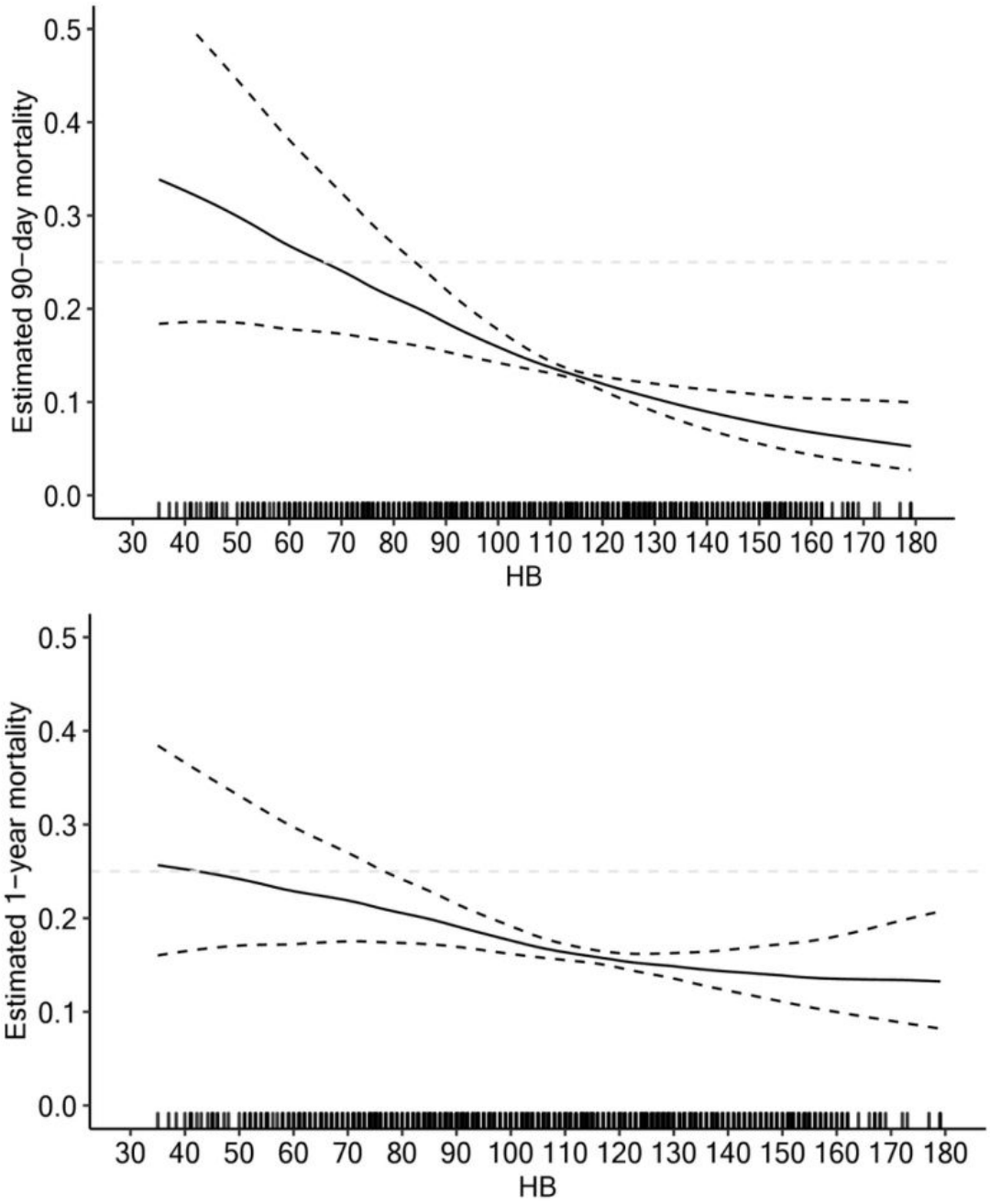

Figure 4

Curves were taken from estimators of GAM. Solid lines are predictions from a generalized additive model and dashed lines represent the corresponding 95% confidence intervals. Adjusted for age, gender, etiology, ascites, HE, infection, TB, INR, CR, ALB, ALT, AST, WBC, PLT, Na

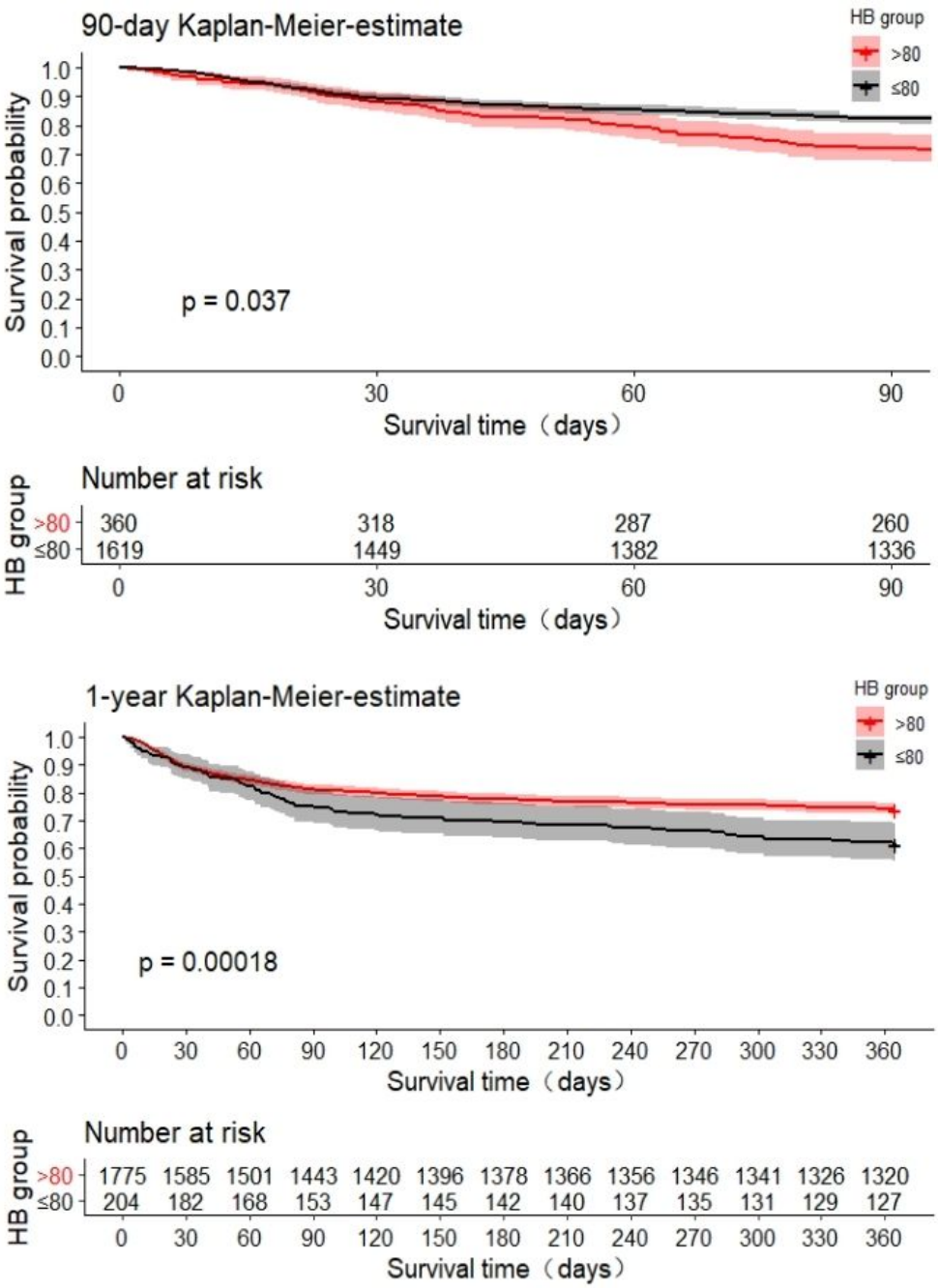

Figure 5

Comparisons of survival curve between cirrhotic patients with or without sever anemia. The cumulative 90-day and 1-year survival across the groups was compared using the Log–rank test.