# Lower serum bilirubin is a risk factor for cardiovascular mortality in maintenance hemodialysis patients: a retrospective cohort study

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# Abstract

**Background:** Although recent studies showed serum bilirubin, an endogenous antioxidant, is protective against cardiovascular diseases, cancer, and diabetic complications, less information is available its association with cardiovascular mortality in hemodialysis patients. This study aimed to investigate the relationship between serum bilirubin and the cardiovascular mortality in maintenance hemodialysis patients.

**Methods:** This retrospective study included 284 chronic hemodialysis patients who started hemodialysis between January 01, 2003, and May 30, 2019. The endpoint was cardiovascular death and all-cause death. A Cox proportional hazards model was used to evaluate the risk factors for cardiovascular death in the maintenance hemodialysis. The cardiovascular mortality was evaluated by Kaplan-Meier analysis.

**Results:** Up to 2019, the median follow-up time was 53 months. In Kaplan–Meier analysis curves, the risk of cardiovascular death in the patients with serum indirect bilirubin (IBIL) levels□3.0 μmol/L was significantly higher than those with serum IBIL levels≥3.0 μmol/L($p$ =0.045). In multivariate Cox regression analysis, the risk of cardiovascular mortality in patients with serum IBIL levels ≥3.0 μmol/L was 0.556 times the risk in patients with serum IBIL levels□3.0 μmol/L (Hazard ratio=0.556, 95% confidence interval 0.334~0.926, $p$ =0.024). However, there was no significant association between serum IBIL and all-cause mortality ($p$ =0.269).

**Conclusions:** Our findings suggest that low serum IBIL level is independently associated with high risk of cardiovascular death in maintenance hemodialysis patients.


# Background

Between 40% and 50% of deaths among patients with end stage renal disease (ESRD) are due to cardiovascular causes, and the risk of cardiovascular mortality in patients with ESRD is over 10-fold of the risk observed in the general population[1–3]. Therefore, it is an important clinical priority to find novel and innovative biomarker and potential therapeutic target to reduce cardiovascular mortality and improve clinical outcomes in ESRD.

Bilirubin possesses potent antioxidant[4–6], anti-inflammatory[7, 8], and possibly lipid-lowering properties[9, 10]. Recent clinical studies show mildly elevated bilirubin is associated with protection from kidney damage and dysfunction in chronic kidney disease patients[11–13], and low serum total bilirubin levels are also associated with the loss of residual kidney function in peritoneal dialysis patients[14]. Furthermore, similar inverse associations have now been shown between serum bilirubin concentrations and coronary artery disease[15], coronary heart disease[16], peripheral vascular disease[17], and stroke[18]. Moreover, mildly elevated bilirubin concentrations may protect against cardiovascular and total death[19–21], whereas other research indicates that higher levels of bilirubin have increased or null associations with cardiovascular diseases (CVD)[22, 23].

In light of the ongoing debate on the potential value of serum bilirubin levels in CVD risk prevention, we conducted a retrospective cohort study to evaluate the relationship between serum bilirubin and cardiovascular death and all-course death in maintenance hemodialysis (MHD) patients.

# Methods

### Study subjects

The protocol of this retrospective single-center cohort study was approved by the Ethics Committee of Shanghai Fifth People's Hospital of Fudan University. Written informed consent was obtained from each patient prior to study participation. The study population consists of 284 patients aged over 18 years with ESRD receiving maintenance hemodialysis for at least 3 months from Shanghai Fifth People's Hospital. Study subjects were recruited between January 01, 2003, and May 30, 2019. The median follow-up time was 53 months (30 ~ 86 months). All of the patients were subjected to a standard bicarbonate dialysis session. Hemodialysis was performed 3 times weekly using single-use dialyzers with a membrane surface area of 1.6 to 1.7 m2. Exclusion criteria were (1) weekly dialysis for less than 12 hours; (2) baseline conditions of malignancy, infectious disease, or sepsis; (3) patients whose serum total bilirubin, alanine aminotransferase (ALT), or aspartate aminotransferase (AST) levels were > 1.5 times the upper limit of normal value; (4) patients with missing bilirubin levels (Fig. 1).

The endpoint was cardiovascular death and all-cause death. The cardiovascular death was defined as death due to ischemic heart disease, cerebrovascular disease, hypertensive heart disease, peripheral vascular disease, cardiac arrhythmia, cardiac arrest, heart failure. All-cause death was defined as any death [24]. If the patients died in any hospital, death certificates were referred to for the exact cause of death, and if death occurred outside a hospital, experts would obtain a consensus about the cause of death after a comprehensive consideration of the history, recent situations, signs, and symptoms before and after death from the patient's medical records in our division and descriptions provided by family members.

### Clinical and biochemical assessment

Baseline demographic data, clinical data and biochemical parameters were collected at the initiation of hemodialysis therapy. The baseline blood samples of each patient were collected at the initiation of hemodialysis therapy. These data were complemented by clinical assessment of blood pressure (BP), and fasting blood glucose. Diabetes was diagnosed on the basis of the World Health Organization criteria. Hypertension was defined as BP≥140/90 mmHg and/or the use of antihypertensive medication. We used the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) creatinine equation to calculate baseline estimated glomerular filtration rate(eGFR).

### Statistical analysis

The Kolmogorov-Smirnov test was used to check the normality of the data distribution. Data are expressed as means ± SD for continuous parametric data, medians and interquartile ranges for continuous nonparametric data, and frequencies for categorical data. Cumulative survival curve for the cardiovascular mortality was generated using the Kaplan–Meier method. The Cox proportional hazards model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of cardiovascular mortality, initially without adjustment and subsequently adjusting for clinical parameters. For all tests, $p$ values of $< 0.05$ were considered statistically significant. All statistical analyses were performed using IBM SPSS software (SPSS Inc., Chicago, IL).

## Results

The study population consists of 284 MHD patients, so the patients were divided into the following two groups according to the median indirect bilirubin (IBIL) value (3.0 μmol/L): I1 (low IBIL group; ≤3.0 μmol/L) and I2 (high IBIL group; ≥3.0 μmol/L). And there were 139 patients in the I1 group and 145 patients in the I2 group. In the I1 group, 38 of the 139 patients cardiovascular death occurred, while in the I2 group, 34 of the 154 patients cardiovascular death occurred. The baseline characteristics and clinical outcomes of the study population are displayed in Table 1. In the

I2 group, serum total bilirubin (TBIL) direct bilirubin (DBIL), IBIL and serum albumin and calcium value were higher; follow-up time was longer, compared with those in the I1 group.

Table 1
Baseline characteristics of chronic hemodialysis patients classified by the serum indirect bilirubin median value

| characteristics of patients | All Participants | Serum indirect bilirubin concentration | | *p* Value |
|---|---|---|---|---|
| | | I1: ⍰3 μmol/L | I2: ≥3μmol/L | |
| N | 284 | 139 | 145 | - |
| Men, n(%) | 162(57.0%) | 84(60.4%) | 77(53.8%) | 0.259 |
| Age (yr) | 59.82 ± 13.93 | 58.79 ± 13.83 | 60.80 ± 14.01 | 0.222 |
| Follow time(months) | 53(30–86) | 49(30–78) | 63(30–103) | 0.018 |
| Hypertension | 99(34.9%) | 50(36.0%) | 49(33.8%) | 0.774 |
| Diabetes mellitus | 82(28.9%) | 44(31.7%) | 38(26.2%) | 0.595 |
| Laboratory values | | | | |
| Hemoglobin (g/L) | 85(70–100) | 83(65–99) | 86(71.5–101) | 0.132 |
| Albumin (g/dL) | 36.2(32. 2-40.3) | 34.9(31.4–38.6) | 37.7(33.6–41.3) | 0.001 |
| Creatinine (μmol/L) | 769(626–1009) | 755(618–968) | 774(628–1019) | 0.647 |
| Uric acid(μmol/L) | 475(376–577) | 476(379–599) | 474(366–568) | 0.324 |
| eGFR, mL/min/1.73 $m^2$ | 5.32(4.00-7.05) | 5.37(3.96–7.31) | 5.26(4.11–7.01) | 0.934 |
| Total cholesterol (mg/dL) | 3.92 (3.31–4.78) | 3.93(3.22–4.66) | 3.90(3.37–4.82) | 0.330 |
| LDL-C (mg/dL) | 2.30(1.72–2.93) | 2.32(1.68–2.99) | 2.26(1.81–2.89) | 0.660 |
| CRP (mg/dL) | 3.4 (1–17) | 4.9(1–24) | 3(1–14) | 0.326 |
| Calcium(mmol/L) | 2.04(1.85–2.20) | 2.02(1.82–2.15) | 2.10(1.86–2.25) | 0.035 |
| Phosphorus(mmol/L) | 1.83(1.48–2.29) | 1.90(1.52–2.30) | 1.74(1.43–2.28) | 0.127 |
| Magnesium (mmol/L) | 0.87(1.00-1.15) | 1.00(0.87–1.13) | 1.00(0.87–1.16) | 0.678 |
| TBIL(μmol/L) | 5.4 (1.9-7.0) | 3.9(3.1–4.8) | 6.9(5.6–8.3) | < 0.001 |
| DBIL(μmol/L) | 2.2 (1.6-3.0) | 2.0(1.5–2.7) | 2.3(1.8–3.1) | 0.029 |
| IBIL(μmol/L) | 3.0 (1.9–4.2) | 1.9(1.3–2.4) | 4.0(3.4–5.7) | < 0.001 |
| ALT (U/L) | 11(7–18) | 11(7–18) | 11(7-18.8) | 0.503 |
| AST (U/L) | 15(11–20) | 15(11–21) | 15(11–19) | 0.714 |
| Parathyroid hormone(pg/ml) | 92.4(30.4–228) | 82.9(29.7–222) | 93.3(31–228) | 0.759 |
| Clinical outcomes | | | | |
| CVD death | 72(25.4%) | 38(27.3%) | 34(23.4%) | 0.451 |

Values expressed as mean ± standard deviation, median, percentage or median (interquartile range).

eGFR, estimated glomerular filtration rate; LDL-C, low-density lipoprotein- cholesterol; CRP, C-reactive protein; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine amino-transferase; AST, aspartate amino-transferase; CVD, cardiovascular diseases;

| characteristics of patients | All Participants | Serum indirect bilirubin concentration | | *p* Value |
|---|---|---|---|---|
| All-cause death | 128(45.1%) | 59(42.4%) | 69(47.6%) | 0.384 |
| Values expressed as mean ± standard deviation, median, percentage or median (interquartile range). | | | | |
| eGFR, estimated glomerular filtration rate; LDL-C, low-density lipoprotein- cholesterol; CRP, C-reactive protein; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine amino-transferase; AST, aspartate amino-transferase; CVD, cardiovascular diseases; | | | | |

During the 53 months (30 ~ 86 months) observation period, the all-cause mortality rate was 45.1% and 72 deaths (25.4%) were CVD -related (Table 1). In Kaplan–Meier analysis curves for the cardiovascular mortality among 284 MHD patients, the risk of cardiovascular mortality in the I1 group with serum IBIL levels□3.0 μmol/L was significantly higher than those in the I2 group with serum IBIL levels ≥ 3.0 μmol/L ( $p = 0.045$, Fig. 2). However, there was no significant difference in the risk of all-cause mortality between the two groups($p$□0.05).

We converted serum IBIL into binary variables according to median values when performing the Cox regression analysis. In univariate Cox regression analysis, age, serum IBIL, ALT and AST were the risk factors for cardiovascular death ($p$□0.05,Table 2). Table 3 shows unadjusted and multivariable-adjusted HRs with 95% CIs according to baseline serum IBIL concentration as a continuous variable and binary variable. In the multivariate Cox regression analysis, compared with the I1 group with serum IBIL levels□3.0 μmol/L, the fully adjusted HRs in the I2 group with serum IBIL levels ≥ 3.0 μmol/L for cardiovascular mortality was 0.556( 95% CI 0.334 ~ 0.926, $p = 0.024$), after adjustment for age, serum uric acid, hemoglobin, serum albumin, eGFR, serum calcium, serum phosphorus, AST and ALT (Table 3). In the same multivariable-adjusted model, age was also the independent risk factor for cardiovascular death (HRs = 1.041, 95% CI 1.020□1.062, $p < 0.001$, Fig. 3). Consistently, there was no significant association between serum uric acid, eGFR, serum albumin, hemoglobin, serum calcium, serum phosphorus, DBIL, TBIL, ALT or AST and cardiovascular death (Fig. 3). However, there was no significant association between serum IBIL and all-cause mortality (HRs = 0.818, 95% CI 0.574 ~ 1.167, $p = 0.269$).

Table 2
The relationship between cardiovascular death and the baseline parameters using the univariate Cox proportional hazards analysis in maintenance hemodialysis patients

| Predictors | Univariate analysis | | | |
|---|---|---|---|---|
| | Coefficient | HRs | 95%CI | *p* value |
| Age | 0.040 | 1.041 | 1.021–1.061 | < 0.001 |
| Male | 0.184 | 1.202 | 0.751–1.924 | 0.442 |
| Hypertension | 0.164 | 0.849 | 0.457–1.578 | 0.605 |
| Diabetes | 0.384 | 1.468 | 0.358–6.028 | 0.594 |
| eGFR | 0.073 | 1.075 | 0.982–1.177 | 0.118 |
| Albumin | -0.026 | 0.974 | 0.939–1.010 | 0.159 |
| Hemoglobin | 0.000 | 1.000 | 0.988–1.013 | 0.945 |
| Uric acid | 0.001 | 1.001 | 1.000–1.003 | 0.127 |
| CRP | 0.000 | 1.000 | 0.989–1.010 | 0.975 |
| ALT | 0.002 | 1.002 | 1.001–1.004 | 0.004 |
| AST | 0.002 | 1.002 | 1.001–1.003 | 0.007 |
| Total bilirubin | -0.059 | 0.942 | 0.859–1.035 | 0.213 |
| Direct bilirubin | 0.111 | 1.117 | 0.977–1.278 | 0.106 |
| Indirect bilirubin | -0.484 | 0.617 | 0.383–0.993 | 0.047 |
| Total cholesterol | 0.009 | 1.010 | 0.826–1.233 | 0.926 |
| LDL-C | 0.037 | 1.038 | 0.824–1.308 | 0.752 |
| Calcium | -0.311 | 0.733 | 0.356–1.508 | 0.399 |
| Phosphorus | 0.133 | 1.142 | 0.799–1.633 | 0.465 |
| Parathyroid hormone | 0.000 | 1.000 | 0.998–1.001 | 0.628 |

HRs, hazard ratios; 95%CI, 95% confidence interval.

CRP, C-reactive protein; ALT, alanine amino-transferase; AST, aspartate amino-transferase; eGFR, estimated glomerular filtration rate; LDL-C, low-density lipoprotein- cholesterol;

Table 3
The relationship between cardiovascular death and the baseline serum indirect bilirubin by multiple COX analysis

| | Binary variable | | | | Continuous variable | | | |
|---|---|---|---|---|---|---|---|---|
| | Coefficient | HRs | 95%CI | *p* value | Coefficient | HRs | 95%CI | *p* value |
| Unadjusted | -0.484 | 0.617 | 0.383 ~ 0.933 | 0.047 | -0.161 | 0.852 | 0.751 ~ 0.966 | 0.012 |
| Model 1 [a] | -0.626 | 0.535 | 0.330 ~ 0.867 | 0.011 | -0.199 | 0.819 | 0.717 ~ 0.936 | 0.003 |
| Model 2 [b] | -0.569 | 0.566 | 0.347 ~ 0.925 | 0.023 | -0.192 | 0.825 | 0.723 ~ 0.943 | 0.005 |
| Model 3 [c] | -0.507 | 0.602 | 0.369 ~ 0.983 | 0.043 | -0.171 | 0.843 | 0.737 ~ 0.964 | 0.012 |
| Model 4 [d] | -0.604 | 0.547 | 0.326 ~ 0.916 | 0.022 | -0.221 | 0.801 | 0.691 ~ 0.929 | 0.003 |

CI, confidence interval; HRs, hazard ratios.

[a] Adjusted for age

[b] Adjusted for model 1 covariates and hemoglobin, serum albumin, estimated glomerular filtration rate

[c] Adjusted for model 2 covariates and serum uric acid

[d] Adjusted for model 3 covariates and serum calcium, serum phosphorus, alanine amino-transferase, aspartate amino-transferase

# Discussion

Our study demonstrated individuals with elevated serum IBIL concentrations are at reduced risk of cardiovascular death among MHD patients followed for 53 months. This relationship also remained significant even after adjusting for potential confounding factors, including age, serum uric acid, hemoglobin, serum albumin, eGFR, serum calcium, serum phosphorus. However, there was no relationship between circulation IBIL and all-cause mortality in this study.

Our finding that a high serum IBIL level was a protective factor for cardiovascular death in MHD patients is consistent with the results in previous studies. A cohort study followed for 12 years comprised 661 chronic hemodialysis patients demonstrated that individuals with bilirubin in the upper tertile had an adjusted hazard ratio of 0.32 for cardiovascular event, compared with those in the lower tertile [25]. Additionally, in 1,419 patients with angina pectoris undergoing percutaneous coronary intervention, those with higher bilirubin concentrations (≥8.4 μmol/L) experienced significantly fewer long-term MACEs (cardiac death, myocardial infarction, target-vessel revascularization, or unstable angina pectoris/heart failure, over 2.4-yr follow up) than patients with lower bilirubin concentrations[26]. Moreover, investigation of 3,316 Ludwigshafen Risk and Cardiovascular Health Study

participants revealed that increased bilirubin predicted lower overall mortality over a period of 10.4 yr[27]. Furthermore, a study included 2936 subjects followed for 5.4 years showed that higher serum concentrations of bilirubin were associated with a decreased risk of developing cardiovascular death in asymptomatic diabetic patients [20]. However, few studies drew opposite conclusions. A large retrospective study investigating 1111 patients indicated that patients with ST-segment elevation myocardial infarction with higher bilirubin undergoing percutaneous coronary intervention and stent placement had increased MACE and rate of cardiac death during their hospital admission[28]. The discrepancies in ethnicity and inclusion criteria may be confounding factors that caused these different results.

The high risk of cardiovascular morbidity and mortality in MHD individuals is associated with a high prevalence of traditional risk factors for cardiovascular disease (hypertension, diabetes, dyslipidemia). Apart from these risk factors, a series of nontraditional risk factors[29, 30] including calcium and phosphate abnormalities, oxidative stress, inflammation, and malnutrition may render ESRD patients more prone to develop excess risks of cardiovascular death. The recognition of bilirubin as an important endogenous anti-inflammatory and antioxidant molecule has increased in recent decades. Bilirubin affects atherosclerosis by several inhibiting mechanisms, including low-density lipoprotein oxidation, vascular smooth muscle cell proliferation, and endothelial dysfunction [31]. Additionally, elevated bilirubin concentrations are associated with decreased oxidative stress status and augmented endothelium dependent vasodilation in male gilbert syndrome subjects[32]. Moreover, in spontaneously hypertensive rats, the administration of hemin for 3 months elevated bilirubin levels and total antioxidant capacity and reduced left ventricular hypertrophy, hypertension, ventricular phospholipase C activity, circulating aldosterone, and urinary excretion of oxidized lipids[33]. Furthermore, lipid soluble antioxidant bilirubin prevents the oxidation of cardiolipin and decreases the infarct size in the heart during ischemia[34]. Considered together, the antioxidative characteristic of bilirubin might protect against the cardiovascular death.

The present study has some limitations. First, this was a retrospective study, and therefore, larger clinical prospective studies are required to validate our results. Second, the relatively small sample size, single-center study might have selection bias and reduce the statistical power. Third, potential mechanism behind the relationship between serum bilirubin and cardiovascular mortality in MHD individuals was not clarified, and thus, persistent uncertainty is a call to arms for scientists and researchers in this neglected area.

# Conclusion

A lower bilirubin level increased the risk of cardiovascular death in MHD patients independent of established CVD risk factors. If our findings are further confirmed by future studies, routine measurements of bilirubin could help identify those patients at high risk of cardiovascular death, and it is hoped that new therapies will be developed to prevent the mortality from CVD in MHD individuals.

# Abbreviations

ESRD: end stage renal disease; CVD: cardiovascular diseases ; MHD: maintenance hemodialysis; ALT: alanine aminotransferase; AST: aspartate aminotransferase ;BP: blood pressure; CKD-EPI : Chronic Kidney Disease Epidemiology Collaboration; eGFR :estimated glomerular filtration rate; HRs: hazard ratios; CIs :confidence intervals; IBIL: indirect bilirubin; TBIL: total bilirubin; DBIL: direct bilirubin; MACEs: cardiac death, myocardial infarction, target-vessel revascularization, or unstable angina pectoris/heart failure

# Declarations


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### Availability of data and materials

The datasets created during and/or analyzed during the current study available from the corresponding author on reasonable request.

### Author's contributions

JN conceived the study and revised the manuscript. YC and PZ collected the data with the help of WF, HL, XZ. YC and PZ contributed to the statistical analysis. YC drafted the manuscript. All the authors have read and approved the manuscript for submission.

### Ethics approval and consent to participate

All subjects provided written informed consents. The study was approved by the ethics committee of Shanghai Fifth People's Hospital of Fudan University. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

### Competing interests

The authors declare that they have no competing interests

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# Figures

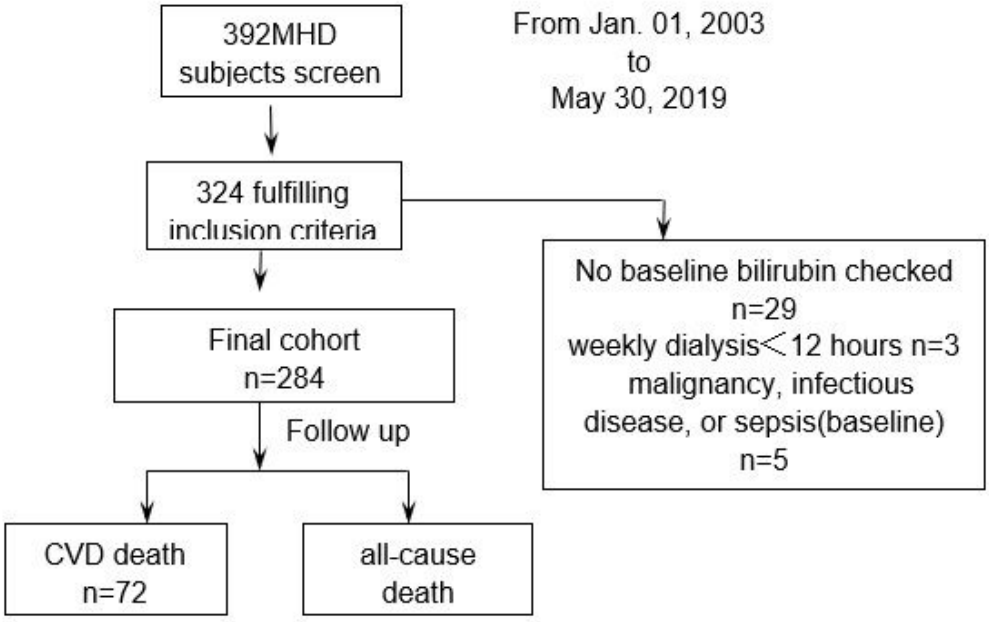



**Figure 1**

Study flow

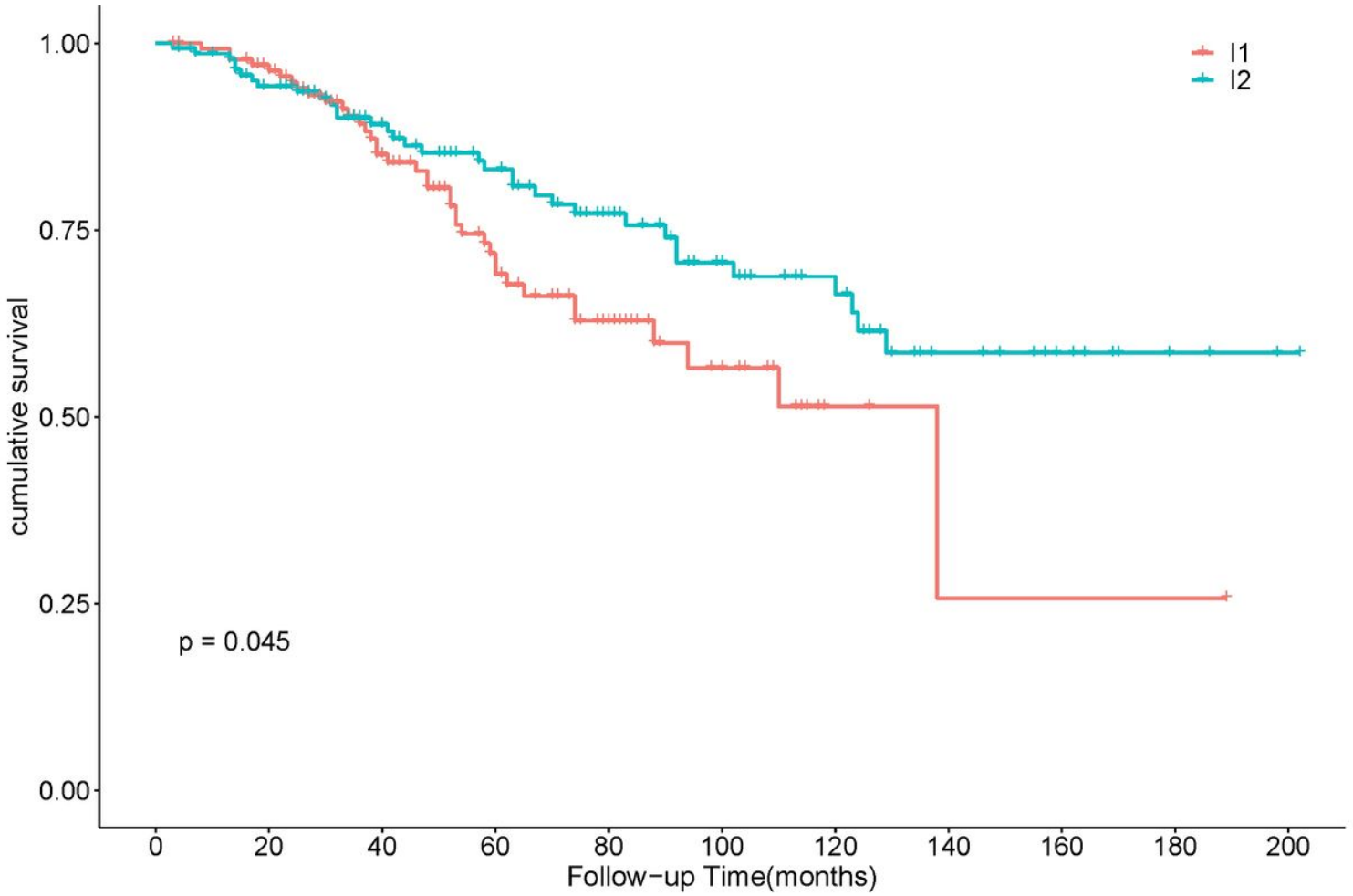
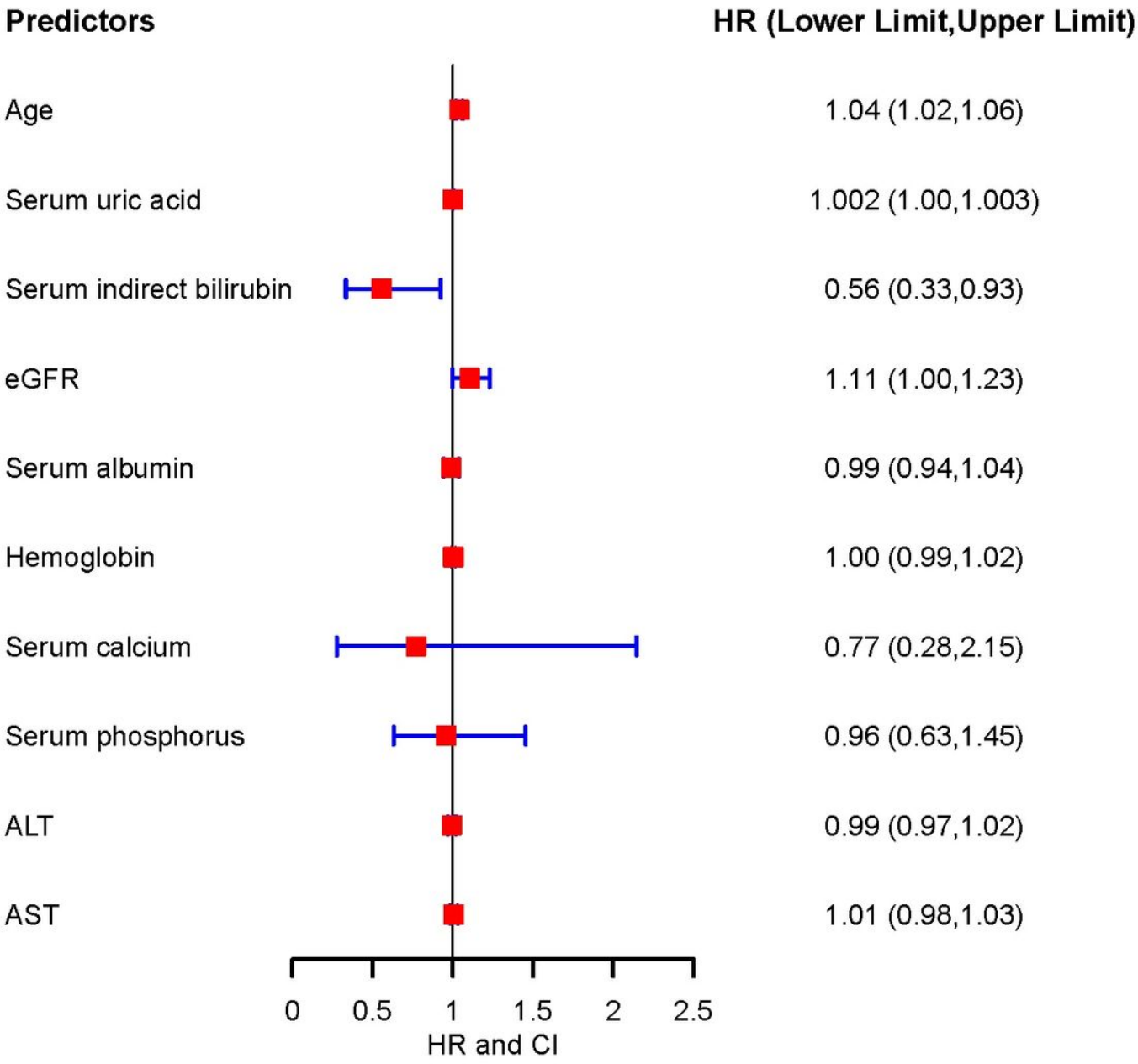



**Figure 2**

Kaplan-Meier analysis curves for cardiovascular mortality in two groups (I1 group with serum IBIL□3.0 μmol/L, I2 group with serum IBIL≥3.0 μmol/L . The log-rank test showed a significant difference between the two groups (p=0.045). Symbols are: I1 □□□ ; I2 □




## Figure 3

The relationship between cardiovascular death and the baseline parameters using the multivariate Cox proportional hazards analysis in maintenance hemodialysis patients□serum IBIL concentration as binary variable□