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Associations between serum levels of liver function biomarkers and all-cause and cause-specific mortality: a prospective cohort study

Shunhu Ling[1], Haiping Diao[2], Guangbing Lu[3] and Luhua Shi[4*]

Abstract

Background The liver plays critical roles in human health. Circulating level of liver function biomarkers may associate with the long-term and short-term mortality in general population.

Methods We used data from US National Health and Nutrition Examination Survey 1988–94 and 1999–2014. People aged ≥ 20 years with measured serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), total bilirubin (TB), and albumin (ALB) at baseline were included. All-cause and cause-specific mortality was identified from the National Death Index through 31 December 2015. Additive Cox regression models were applied to assess the correlation patterns between the serum level of these analytes and mortality risk.

Results A total of 44,508 participants were included; among them, 9,721 deaths occurred during a mean follow-up of 12.5 years. A "J-shaped" correlation was found between serum levels of ALT, AST, and TB and all-cause mortality. The risk of mortality monotonically increased with increasing GGT and ALP levels when their levels exceeded the valley points. A "L-shaped" correlation was found between the serum level of ALB and all-cause mortality. The correlation patterns were comparable among deaths from different causes and were consistent in subgroup and sensitivity analyses. While the integration of all six liver function biomarkers did not yield an optimal predictive performance for mortality (area under ROC curve = 0.706), it demonstrated a significantly better performance compared to any single biomarker..

Conclusion Circulating liver function biomarkers showed diverse nonlinear correlations with mortality and may be utilized as part of a screening process to help identify individuals who may be at elevated risk of mortality.

Keywords Mortality, Liver enzyme, Prediction, Liver function, Cohort study, NHANES

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Introduction

The liver is a dynamic organ that plays critical roles in many physiological processes, including the regulation of the endocrine system, systemic glucose, and lipid metabolism and the control of immune responses to local and systemic agents as well as disease tolerance [1–3]. Liver dysfunction has long been demonstrated to be associated with a set of serious clinical outcomes [4–10]. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), total bilirubin (TB), and albumin (ALB) are the most commonly used biomarkers to measure liver function in clinical practice. Liver enzymes exert their biological function via different mechanisms. ALT and AST are found abundantly within hepatocytes, and they catalyse the transfer of amino groups to generate products of gluconeogenesis and amino acid metabolism [11]. GGT is responsible for the extracellular catabolism of glutathione, a thiol antioxidant that protects specific components of mammalian cells from damage by reactive oxygen species [12]. ALP is a hydrolase enzyme that catalyses the hydrolysis of inorganic pyrophosphate, which is a vascular calcification inhibitor. Serum TB is a normal metabolite of haem and has been used as a marker of liver and haematopoietic diseases [13]. ALB is produced by the liver and is pivotal for maintaining the oncotic pressure needed for proper distribution of body fluids between blood vessels and body tissues [14].

Several epidemiological studies have reported associations of serum levels of liver enzymes with diabetes, cardiovascular disease, and mortality [12, 15–18]. For example, a monotonic increasing trend has been detected between cardiovascular disease-related mortality and GGT levels, suggesting that elevation of GGT levels, even within the normal medical referent interval, might have an adverse impact on long-term health [18]. Similar results have also been reported for other liver enzymes, although their relationships with mortality showed different patterns [17]. These results indicated that serum liver function biomarkers, including liver enzymes, TB, and ALB, might serve as independent predictors of long-term mortality in the general population.

Although some cohort studies have been conducted on this issue, these studies were mostly conducted in selected populations, such as in the elderly, and were mainly focused on individual liver enzymes and did not take into account their interactions [12, 15, 19]. In the current study, we aimed to investigate the associations of the serum levels of six liver function biomarkers with all-cause and cause-specific mortality. We also tested the performance of these biomarkers, individually and in combination, in the prediction of mortality.

Materials and methods

Study population

We used data from the US National Health and Nutrition Examination Survey (NHANES) from 1988–2014. Details of NHANES have been described elsewhere [20]. Briefly, NHANES used a complex, multistage, probability sampling method to collect nationally representative health-related data on the US population [21]. The NHANES interview included demographic, socioeconomic, dietary, and health-related questions. The examination component consists of medical, dental, and physiological measurements, as well as laboratory tests administered by highly trained medical personnel. NHANES was conducted periodically before 1999 (namely, NHANES III: 1988–1994) and on a continuous basis thereafter (namely, continuous NHANES: 1999–2014).

In this analysis, we included only participants aged ≥ 20 years at the baseline survey, which resulted in 18,825 and 43,510 participants from NHANES III and continuous NHANES, respectively (Fig. 1). We used a series of exclusion criteria to filter the participants: 1) participants who were infected with hepatitis B and/or C viruses or missing for this information were excluded; 2) participants who were missing data for mortality status were excluded; 3) to avoid the biases introduced by transient increase or decrease in serum levels of liver function biomarkers, we also excluded participants who had outlier or missing information (Fig. 1). Finally, a total of 43,838; 43,963; 41,044; 44,112; 44,162; and 44,215 participants were eligible for analyses of ALT, AST, GGT, ALP, TB, and ALB, respectively.

Assessments of liver function biomarkers and covariates

Each analyte included in the current study is described separately within Supplemental Box 1. We also collected data on covariates from NHANES, including demographical characteristics (e.g., sex, ethnicity, education, family income-poverty ratio, and marital status), smoking status, alcohol consumption, physical activity level, and self-reported health status and disease histories. Alcohol consumption was categorized as never, low to moderate, and excessive. Never drinkers replied no to the question: "In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?" Low-to-moderate alcohol consumption was defined as ≤ 1 drink daily among women and ≤ 2 drinks daily among men; otherwise, it was defined as excessive drinking [22]. Standardized measurements of height, weight, and systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained. Body mass index (BMI) was calculated

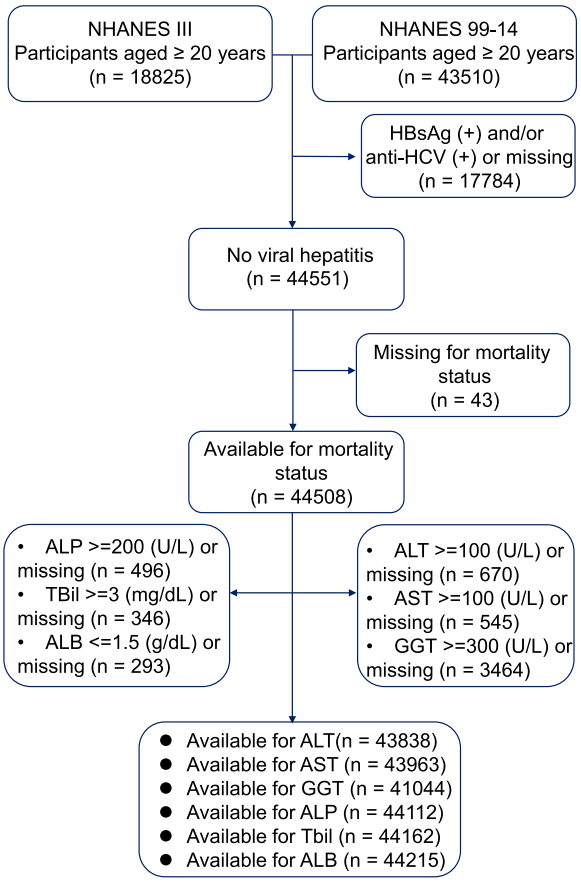

Fig. 1 The derivations of NHANES samples for analysis of serum levels of liver function biomarkers and mortality. HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; TB, total bilirubin; ALB, albumin; HR, hazard ratio; LFRS, liver function risk score

as weight (kg) divided by the square of height (m^2) and then categorized as underweight (< 18.5), normal (18.5–24.9), overweight (25.0–29.9), or obese (≥ 30.0). High blood pressure was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg. Type II diabetes was defined as fasting plasma glucose ≥ 126 mg/dL (7 mmol/L), self-reported history or the use of oral hypoglycaemics or insulin.

Mortality data ascertainment

We ascertained mortality status by probabilistic matching to the National Death Index through 31 December 2015 using a unique study identifier [23]. We classified causes of death according to ICD-10 codes. Primary outcomes for our study were mortality from all causes, heart and cerebrovascular diseases (ICD-10 codes: I00-I09, I11, I13, I20-I51, and I60-I69), malignant neoplasms (ICD-10 codes: C00-C97), and other causes.

Statistical analysis

Based on the previously reported nonlinear associations of serum liver function biomarker levels with mortality [15, 17, 24], we used an approach incorporating nonlinear effects into the Cox model to express the log hazard as an additive function (Supplemental Box 2) [25]. Considering the effective sample size, we constructed 9 covariate Cox models (i.e., sex, age, BMI, smoking, income, education, ethnicity, self-reported health, and the serum levels of each of the liver function biomarkers) to separately assess the correlations of serum levels of six analytes with mortality. We applied the following procedure to obtain the optimal multivariable degrees of freedom (*dfs*) that minimize the model's Akaike Information Criterion (AIC) score: 1) We set the maximum value for the *dfs* for each continuous covariate (i.e., serum levels of liver function biomarkers, age, and BMI values). Here, we set the max *dfs* of 20, 10, and 10 for the three categories of continuous variables (serum levels of liver function biomarkers, age, and BMI values, respectively) to thoroughly explore the potential nonlinear associations between these variables and the outcome. 2) We set the minimum value for the *dfs*, namely, 1, for each continuous covariate to represent a possible linear correlation pattern. 3) For each covariate, we created a vector with three values for the *dfs* (i.e., minimum, mean, and maximum). Then, a matrix was created from all combinations of the supplied vectors, the corresponding Cox models were fitted, and the AIC scores were obtained. 4) For each covariate, a vector with two values for the *dfs* was created based on the AIC scores obtained in Step 3 (minimum and mean, or mean and maximum). 5) We repeated steps 3 and 4 a number of times (here, we defined the times = 4), and then chose the *dfs* minimizing the AIC scores. The hazard ratio (HR) along with its 95% confidence interval (CI) were obtained from the additive Cox models and were used to quantify the correlations of our analytes of interest with mortality. The details for smoothing the HR curves are shown in Supplemental Box 3. We also evaluated the importance of each liver function biomarker by adding each biomarker to the Cox regression model and observing the resulting increase in R^2. This method allowed us to quantitatively assess which LFTs most significantly improved the model's predictive accuracy for all-cause mortality.

We calculated a liver function risk score (LFRS) for each participant (details see Supplemental Box 4). Receiver operating characteristic (ROC) curves were drawn based on the LFRS and each biomarker to compare their predictive performance on mortality. We also calculated crude risk scores (CRSs) in terms of the serum level of these analytes at the individual level. For a single biomarker, the value of a level that was not within the medical reference interval was assigned a score

of 1; otherwise, it received a score of 0 (Supplemental Table S1). The medical reference intervals for liver biomarkers are crucial for identifying deviations from normal liver function, as changes in these levels can indicate liver damage, inflammation, or dysfunction. The CRS of each participant, ranging from 0 to 6, therefore provides a preliminary guide for quickly assessing liver function. The ROC curves were then constructed based on the CRSs.

Sensitivity analysis

To ensure the robustness of our results, we conducted a set of sensitivity analyses. First, participants who were followed up for less than 36 months or died within 36 months after the interview were excluded. Second, participants who were diagnosed with diabetes, cardiovascular diseases, or cancers at baseline were excluded. Third, data from NHANES III and the continuous NHANES were analysed separately. Fourth, full models were constructed based on all covariates. Fifth, data from the young (aged < 60 years) and the old (aged ≥ 60 years) groups were analysed separately. Sixth, we redefined the outliers for each biomarker using an objective criterion, i.e., not within the scope of the 1–99% quantile. Participants who were identified as outliers were excluded. Finally, we examined the associations between liver-related biomarkers and all-cause mortality risk for subpopulations with diverse ethnicities. All analyses were conducted using R program (R core team, V 3.5.1), and a two-tailed P value of < 0.05 was considered statistically significant.

Results

Characteristics of study participants

A total of 44,508 participants were included; among them, 9,721 deaths occurred during a mean follow-up of 12.5 years (Table 1). Among the 9,721 people who died during the follow-up, 2,567; 2,044; and 5,100 died from heart and cerebrovascular diseases, cancers, and other causes, respectively. The baseline characteristics were significantly different between people who survived and those who did not. Briefly, non-survivors were older and were more likely to be males and non-Hispanic whites than survivors. Additionally, the proportions of non-survivors receiving less than a high school education, with a low income-poverty ratio, with 0 physical activity sessions per week, and with poor to fair self-reported health were higher. The alcohol consumption status was comparable between the two populations, whereas people were more likely to be never smokers among survivors. Non-survivors were more frequently diagnosed with diabetes, hypertension, cardiovascular disease, and cancer at baseline. For the six liver function biomarkers, the serum levels varied significantly between the survivors and non-survivors (Table 1). For example, the serum levels of ALT and ALB were significantly higher in survivors than in non-survivors. In contrast, serum levels of GGT and ALP were significantly higher in non-survivors than in survivors.

Associations between serum levels of liver function biomarkers and mortality

The distributions of serum levels of liver function biomarkers are shown in Supplemental Figure S1. Based on the 9 covariate additive Cox models, we found nonlinear relations between the six liver function biomarkers and all-cause mortality (Fig. 2). Briefly, a J-shaped correlation was found between the serum levels of ALT, AST, and TB and all-cause mortality, with valley points of 31 U/L, 22 U/L, and 0.9 mg/dL, respectively. Although the correlation of the serum level of ALP with all-cause mortality also showed a "J" shape, the left half of the HR curve (ALP < 50 U/L) was not statistically significant. An upward parabolic correlation was detected between the serum level of GGT and all-cause mortality. An L-shaped correlation was found between the serum level of ALB and all-cause mortality. The valley point was at 4.9 g/dL. The HR values were not statistically significant when the ALB level exceeded this point. Of the total participants, 16.4%, 50.8%, 83.7%, 88.5%, 21.2%, and 3.2% had biomarker levels surpassing the valley points for each of the six biomarkers, respectively (Fig. 2). Figure 2 also presents intervals that highlight the ranges within which biomarker levels do not elevate mortality risk. For example, individuals with ALT levels below 16.9 U/L or above 42.6 U/L exhibited increased mortality. The correlation patterns in both males and females were comparable to those in the whole population, albeit with different valley points (Supplemental Figures S2-3).

Correlation patterns for the serum levels of the six liver function biomarkers were generally comparable between all-cause mortality and cause-specific mortality (Supplemental Figures S4-6). For heart and cerebrovascular disease-related deaths, an approximate "V" or "U" shape correlation was found between the serum level of ALT and mortality. A stronger association was observed in individuals with ALT < 23 U/L. An L-shaped correlation was found between serum TB level and mortality. The HR values were not statistically significant when TB was > 1.0 mg/dL. Similar patterns were also observed in associations of serum levels of ALT and TB with cancer-related deaths (Supplemental Figure S5). For other causes related to mortality, the correlation patterns were highly similar to those observed for all-cause mortality, with the exception of TB. An increased risk of death was detected only among people with a higher TB level (Supplemental

Table 1 The baseline characteristics of study participants in NHANES III and continuous NHANES 1999–2014 according to mortality status until Dec, 31, 2015

Characteristics#	Non-survivors				Survivors	*P* value‡
	All causes (*n* = 9721)	Heart & cerebrovascular diseases (*n* = 2567)	Cancer (*n* = 2044)	Others (*n* = 5110)	(*n* = 34,787)	
Mean age (95% CI), years	65.2(64.6, 65.8)	67.9(67.0, 68.8)	62.3(61.3, 63.3)	65.4(64.6, 66.2)	43.3(42.8, 43.8)	< 0.001
Sex						< 0.001
Men	5200 (53.5)	1414 (55.1)	1146 (56.1)	2640 (51.7)	15,797 (45.4)	
Women	4521 (46.5)	1153 (44.9)	898 (43.9)	2470 (48.3)	18,990 (54.6)	
Ethnicity						< 0.001
Non-Hispanic White	5705 (58.7)	1514 (59.0)	1154 (56.5)	3037 (59.4)	15,582 (44.8)	
Non-Hispanic Black	1746 (18.0)	471 (18.3)	416 (20.4)	859 (16.8)	7373 (21.2)	
Mexican American	1884 (19.4)	493 (19.2)	381 (18.6)	1010 (19.8)	8087 (23.2)	
Others	386 (4.0)	89 (3.5)	93 (4.5)	204 (4.0)	3745 (10.8)	
Education						< 0.001
Less than high school	3260 (33.7)	947 (37.1)	604 (29.7)	1709 (33.6)	4708 (13.6)	
High school or equivalent	3867 (40.0)	970 (38.0)	828 (40.8)	2069 (40.7)	13,976 (40.3)	
College or above	2539 (26.3)	635 (24.9)	599 (29.5)	1305 (25.7)	16,038 (46.2)	
Family income-poverty ratio						< 0.001
0–1.0	1881 (20.3)	503 (20.4)	371 (19.1)	1007 (20.8)	6175 (18.9)	
1.1–3.0	4465 (48.3)	1193 (48.3)	876 (45.0)	2396 (49.6)	13,209 (40.5)	
≥ 3.0	2900 (31.4)	772 (31.3)	699 (35.9)	1429 (29.6)	13,237 (40.6)	
Marital status						< 0.001
Married	5349 (55.6)	1408 (55.2)	1261 (62.5)	2680 (53.1)	19,962 (58.0)	
Single	4269 (44.4)	1141 (44.8)	758 (37.5)	2370 (46.9)	14,479 (42.0)	
BMI categories						0.037
Underweight	221 (2.3)	46 (1.8)	45 (2.2)	130 (2.6)	548 (1.6)	
Normal	3039 (32.2)	791 (31.7)	615 (30.6)	1633 (33.1)	10,740 (31.2)	
Overweight	3498 (37.1)	934 (37.5)	746 (37.2)	1818 (36.8)	11,786 (34.2)	
Obese	2675 (28.4)	722 (29.0)	600 (29.9)	1353 (27.4)	11,399 (33.1)	
Alcohol consumption						0.103
Never	1870 (46.6)	542 (47.8)	195 (36.7)	1019 (49.8)	4786 (42.5)	
Low to moderate	1020 (25.4)	299 (26.4)	227 (28.2)	488 (23.9)	2823 (25.1)	
Excessive	1122 (28.0)	292 (25.8)	282 (35.1)	539 (26.3)	3652 (32.4)	
Smoking status						< 0.001
Never	4117 (42.4)	1105 (43.1)	711 (34.8)	2301 (45.1)	19,309 (55.6)	
Former	3531 (36.4)	991 (38.6)	765 (37.5)	1775 (34.8)	7720 (22.2)	
Current	2065 (21.3)	470 (18.3)	565 (27.7)	1030 (20.2)	7730 (22.2)	
Physical activity level						< 0.001
0 time/week	2318 (32.0)	710 (35.4)	425 (27.5)	1183 (32.1)	4423 (17.4)	
1–2 times/week	2261 (31.2)	589 (29.4)	507 (32.8)	1165 (31.6)	12,274 (48.3)	
≥ 3 times/week	2657 (36.7)	705 (35.2)	613 (39.7)	1339 (36.3)	8725 (34.3)	
Self-reported health status						< 0.001
Very good to excellent	2790 (28.7)	638 (24.9)	705 (34.5)	1447 (28.4)	16,030 (46.1)	
Good	3299 (34.0)	884 (34.5)	699 (34.2)	1716 (33.6)	12,028 (34.6)	
Poor to fair	3620 (37.3)	1043 (40.7)	638 (31.3)	1939 (38.0)	6715 (19.3)	
Diabetes	1266 (17.1)	400 (19.3)	210 (13.3)	656 (17.4)	1448 (6.8)	< 0.001
Hypertension	3588 (41.8)	1112 (48.8)	639 (34.3)	1837 (41.3)	4305 (13.7)	< 0.001
History of diseases						
Cardiovascular diseases	2173 (22.5)	722 (28.3)	322 (15.8)	1129 (22.3)	1974 (5.7)	< 0.001
Cancer*	1302 (13.4)	290 (11.3)	396 (19.4)	616 (12.1)	1914 (5.5)	< 0.001

Table 1 (continued)

Characteristics#	Non-survivors				Survivors	*P* value‡
	All causes (*n* = 9721)	Heart & cerebrovascular diseases (*n* = 2567)	Cancer (*n* = 2044)	Others (*n* = 5110)	(*n* = 34,787)	
Mean ALT (95% CI) (U/L)	21.1 (20.7, 21.6)	20.7 (19.5, 21.8)	20.9 (20.1, 21.7)	21.4 (20.8, 21.9)	24.9 (24.6, 25.2)	< 0.001
Mean AST (95% CI) (U/L)	24.5 (24.1, 24.9)	24.1 (23.4, 24.9)	23.6 (23.0, 24.2)	25.0 (24.4, 25.6)	24.5 (24.2, 24.7)	0.010
Mean GGT (95% CI) (U/L)	35.9 (33.6, 38.3)	34.5 (31.4, 37.5)	34.8 (30.5, 39.1)	37.0 (33.3, 40.7)	27.1 (26.6, 27.7)	< 0.001
Mean ALP (95% CI) (U/L)	82.2 (81.0, 83.3)	84.1 (82.1, 86.1)	80.2 (77.8, 82.6)	82.2 (80.6, 83.9)	68.9 (68.2, 69.6)	< 0.001
Mean TB (95% CI) (mg/dL)	0.69 (0.68, 0.70)	0.68 (0.66,0.70)	0.66 (0.64, 0.69)	0.71 (0.69, 0.72)	0.72 (0.72,0.73)	< 0.001
Mean ALB (95% CI) (g/dL)	4.15 (4.13, 4.17)	4.14 (4.11, 4.17)	4.18 (4.15, 4.21)	4.14 (4.12, 4.16)	4.30 (4.29, 4.31)	< 0.001

Values are numbers (percentage) unless stated otherwise

All estimates accounted for complex survey designs of NHANES

Abbreviations: *BMI* Body mass index, *ALT* Alanine aminotransferase, *AST* Aspartate aminotransferase, *GGT* Gamma-glutamyl transferase, *ALP* Alkaline phosphatase, *TB* Total bilirubin, *ALB* Albumin, *CI* Confidence interval

110, 2641, 449, 602, 29,235, 36, 11,850, 26, 15,641, 4559, and 202 participants had missing information for baseline education level, family income-poverty ratio, marital status, BMI categories, alcohol consumption, smoking status, physical activity level, self-reported health status, fasting plasma glucose (diabetes), systolic blood pressure and/or diastolic blood pressure (hypertension), and cardiovascular diseases and cancer history, respectively

‡ *P* values were derived from comparisons between alive group and all-cause deaths group. For categorical variables, Rao-Scott adjustment was applied in χ^2 test. For continuous variables, analysis of variance was adjusted for sample weights

* cancers other than skin cancer

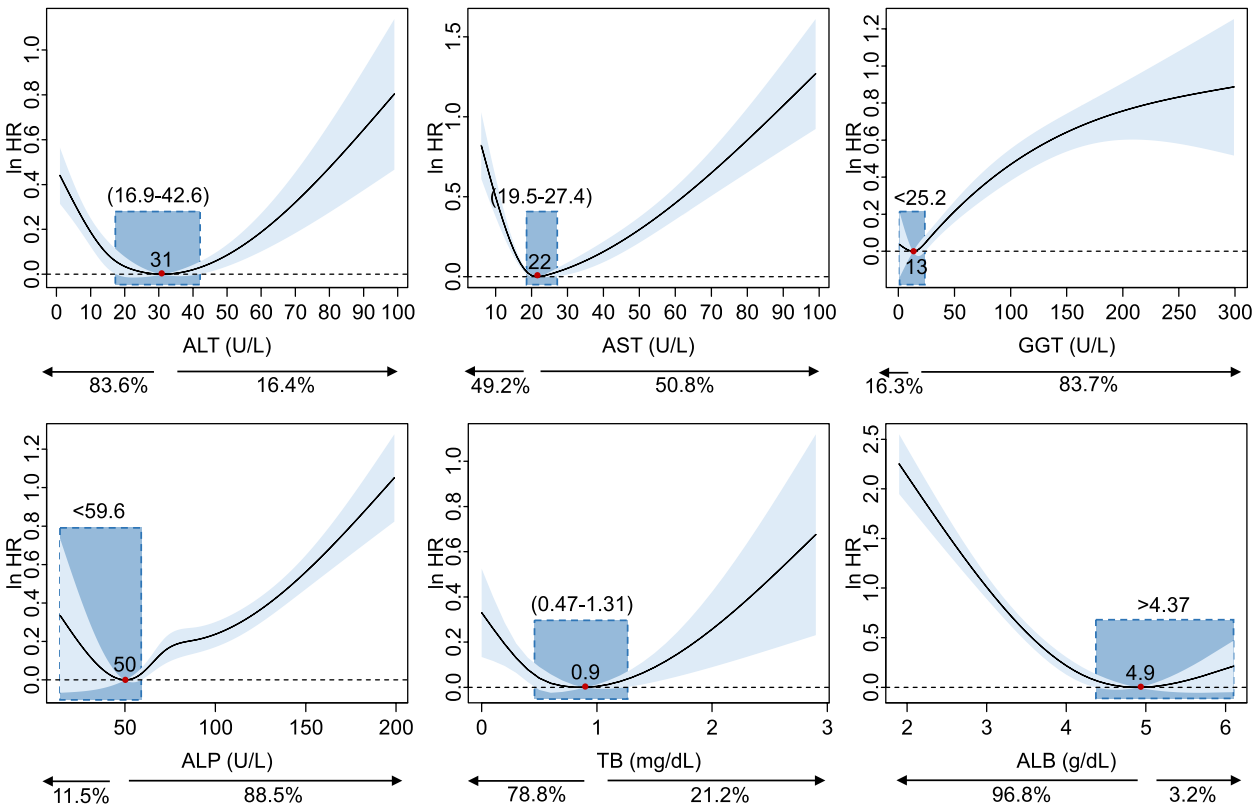

Fig. 2 The dose–response relationships between levels of liver function biomarkers and risk of all-cause mortality. Data were modelled with additive Cox regression models. The degrees of freedom for ALT, AST, GGT, ALP, TB, and ALB were 3, 3, 3, 4, 2, and 2, respectively. The shaded areas are 95% confidence intervals. The vertical axes are on a log scale. The red points in our figures represent the valley values for each biomarker. The steel blue shaded areas, along with their corresponding intervals, indicate the ranges within which biomarker levels do not increase mortality risk. The percentages displayed denote the proportions of participants whose biomarker levels fall below and above these valley points

Figure S6). Supplemental Table S2 and Supplemental Figure S7A show the associations between discrete serum levels of liver function biomarkers and mortality risk.

Predicting mortality using liver function biomarkers

Supplemental Figure S7B illustrates the relative importance of liver function biomarkers for all-cause mortality, with ALB, ALP, and GGT emerging as the most significant predictors, followed by AST, TB, and ALT. We calculated the LFRSs at the individual level by mortality status (Fig. 3A). The LFRS was significantly higher in non-survivors than in survivors ($P=0.001$). We then categorized the LFRSs as high or low by means of their median values. As shown in Fig. 3B, individuals with high LFRSs had a higher risk of all-cause mortality during the long-term follow-up. Similar results were also observed for cause-specific mortality (Supplemental Figures S8-10).

The area under the ROC curve (AUROC) of the six liver function biomarkers and the LFRS are shown in Fig. 3C. At the single biomarker level, the highest AUROC was

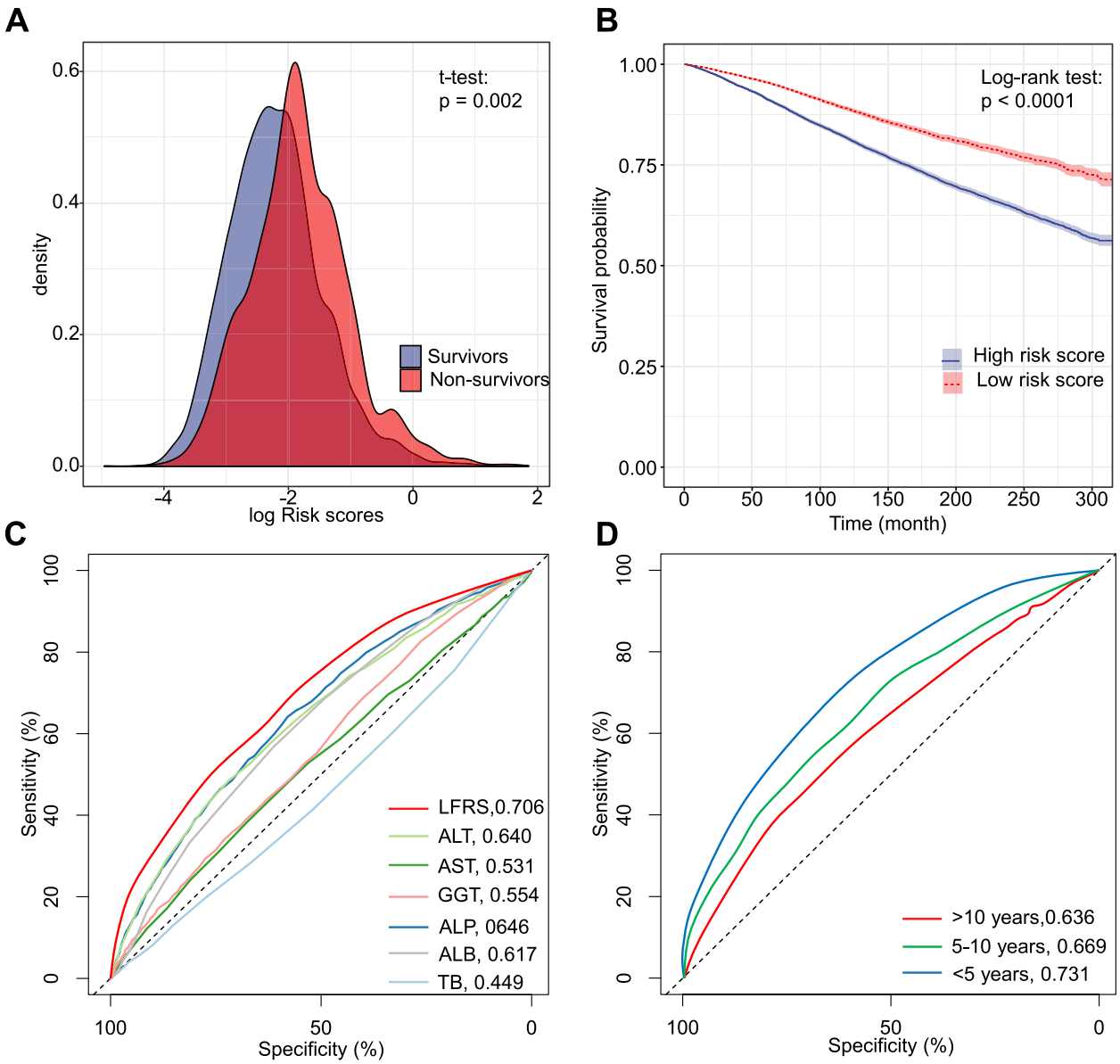

Fig. 3 The predictive performance of liver function risk score on all-cause mortality. **A** The distributions of liver function risk score in people who died and who did not; (**B**) the survival curves of populations with high and low risk scores; (**C**) the predictive performances of six liver function biomarkers and the integrated liver function risk score (LFRS) for all-cause mortality; (**D**) the predictive ability of LFRS for all-cause mortality stratified by years of follow-up

found for ALP (0.646, 95% CI 0.610, 0.676), followed by ALT and ALB. The AUROC increased to 0.706 (95% CI 0.689, 0.722) when using the LFRS to predict mortality status. We also tested the predictive performance of LFRS with the consideration of years of follow-up, i.e., < 5, 5–10, and > 10 years. The AUROC was highest (0.731, 95% CI 0.707, 0.744) in people with a follow-up of < 5 years and decreased to 0.636 (95% CI, 0.621, 0.651) in people with a follow-up of > 10 years (Fig. 3D). The AUROC were 0.689 (95% CI 0.674, 0.703), 0.665 (95% CI 0.636, 0.679), and 0.702 (95% CI 0.685, 0.719) for mortality from heart and cerebrovascular diseases, cancers, and other causes, respectively (Supplemental Figure S11). The AUROC was 0.615 (95% CI 0.586, 0.639) in terms of CRSs.

The results of the sensitivity analyses are presented in Supplemental Figures S12-23. In general, no significant difference was detected between the results shown in the main analyses and those of the sensitivity analyses. Notably, the serum TB level was not associated with the risk of mortality among participants aged < 60 years. A lower level of ALT (< 50 U/L) was also found not to be associated with mortality risk in this population. However, these associations were significant among participants aged ≥ 60 years (Supplemental Figures S17-18). In analyses stratified by different ethnicities, we noted similar correlation patterns between liver-related biomarkers and all-cause mortality risk, albeit with subtle variations in associations across these subpopulations (Supplemental Figures S20-23).

Discussion

To obtain the patterns of the correlations of the levels of liver function biomarkers with the risk of mortality, we used a large and nationally representative sample and advanced modelling strategies. For most analytes, a J- or U-shaped association was detected. Our study also shows the effects of liver function biomarker levels in discrete scales on mortality and suggests the importance of maintaining normal liver function. Additionally, the well-constructed correlation patterns might be indicative of the need to establish more precise medical reference intervals. More importantly, we further tested the prediction performance of these biomarkers on long-term and short-term mortality. Our results show that the combined risk score is more informative for predicting mortality risk and that its prediction performance is superior for short-term mortality.

The associations between serum levels of liver enzymes and mortality have been extensively studied in many prospective cohort studies. However, most studies were conducted in selected populations, such as middle-aged or older participants, and were generally limited to a few enzymes [12, 26–28]. In addition, most previous studies exclusively analysed the discrete effects of the levels of liver enzymes on mortality risk but did not show correlation curves. In the current study, we comprehensively investigated the correlations of the levels of six liver function biomarkers with the risk of mortality using long-term follow-up data and advanced modelling strategies. Our results show distinct correlation patterns between baseline levels of these analytes and mortality and report their effects on mortality on a discrete scale. These results were partially similar to those from previous studies. The association of the level of ALT with mortality is controversial [29–31]. We found a J-shaped correlation between the level of ALT and all-cause and cause-specific mortality. For people with lower and higher serum levels, a 24% to 70% increased risk of mortality was observed. This pattern is consistent with the result of Ruhl et al.'s study, although they reported an inverse J-shaped pattern, suggesting low ALT was associated with higher mortality risk [17]. Likewise, in an elderly population, Koehler et al. found that the increased risk of mortality was observed only in people with lower levels of ALT [12]. These findings align with our observation that ALT levels were significantly higher in participants who were alive compared to those who had died, suggesting that lower ALT levels might be more impactful on mortality risk than elevated ALT levels. By contrast, a large-scale meta-analysis showed that there was no association between the risk of all-cause mortality and levels of ALT [11]. The contradictory results might be ascribed to population variations and merit further investigation. However, our sensitivity analysis across different racial groups revealed similar correlation patterns between liver-related biomarkers and all-cause mortality risk, indicating that the observed associations are consistent in different populations.

AST is not specific for the liver. We also observed a J-shaped correlation pattern for AST in the current study. This result is consistent with some previous reports, although paradoxical results exist [11, 12]. An S-shaped correlation between the level of AST and mortality was also reported previously [32]. Unlike ALT and AST, the correlations of the serum level of GGT with all-cause and cause-specific mortality were more comparable between our results and those from previous studies [28, 33]. The risk of all-cause and cause-specific mortality increased monotonically with increasing levels of GGT. This correlation was consistent among people of different ages and ethnicities [12, 34, 35]. Likewise, a monotonous increase in mortality risk was observed when the ALP level exceeded the valley point. This correlation was also reported elsewhere, irrespective of the age, sex, and ethnicity of the participants [11, 36–38]. In contrast to liver enzymes, studies on the correlation between

serum TB level and mortality have been less commonly assessed among the general population. Here, we report a J-shaped association, although the correlation pattern was not consistent for deaths from different causes and among people of different ages. For example, we observed an L-shaped correlation of TB level with cancer-related mortality, whereas an inverse L-shaped correlation was observed for other cause-related mortality. Previous studies were mostly limited to patients. McCallum et al. reported a significant negative association of serum TB with all-cause and cardiovascular mortality among hypertensive patients [39]. In parallel, Horsfall et al. reported that among patients with respiratory diseases, relatively higher levels of bilirubin were associated with a lower risk of all-cause mortality [40]. However, a contrasting pattern was reported among patients undergoing long-term haemodialysis [41]. The heterogeneous results suggest not only that the correlation of TB level with mortality needs further investigation but also that the potential mechanisms underlying these correlations are far from completely understood. Low serum levels of ALB have long been demonstrated to be associated with a higher risk of death [42, 43]. Here, we observed an "L"-shaped correlation between the serum level of ALB and all-cause mortality. This correlation pattern is consistent with that identified in previous studies [44–46].

Although the correlation patterns between the levels of liver function biomarkers and risk of mortality were to some extent comparable among different populations, the "safe interval" of these biomarkers was significantly heterogeneous. For example, we found that the "safe interval" of ALT was 17–42 U/L, which was similar to the medical reference interval. However, we also observed a right shift in this safe interval among older people (i.e., 20–55 U/L). In another study, the safe interval of ALT was further extended to 20–60 U/L [12]. AST and GGT have a relatively narrow safe interval (20–27 U/L and 0–25 U/L, respectively) in contrast to ALT. This result was also observed in previous studies [12, 28, 39]. For example, the safe interval of GGT was found to be approximately 0–14 U/L in 19,000 construction workers who were followed over 20 years [28]. The safe interval of ALP was approximately 0–60 U/L in our study, which was similar to the findings of previous studies [11, 36]. However, this interval might extend to 0–120 U/L among older people [12]. The safe interval of ALB was approximately > 4.4 g/dL in our study and was consistent among different populations, suggesting that ALB could serve as a stable indicator for mortality irrespective of age, sex, and ethnicity [44].

In our study, we developed an integrated liver function risk score, leveraging the combined predictive power of six key liver function biomarkers. This score represents a novel approach in the assessment of mortality risk. By integrating multiple biomarkers, we were able to capture a more comprehensive picture of liver function than could be discerned through individual markers alone. Notably, the score achieved an area under the ROC curve of 0.706, indicating a significant improvement in mortality prediction compared to single biomarkers. This suggests that the integrated risk score could be a valuable tool in clinical settings for identifying patients at a higher risk of mortality. However, it is important to acknowledge that while this integrated approach shows promise, the practicality and feasibility of its application in routine clinical practice require further evaluation. We envision that with additional refinement and validation, this risk score could become an important part of preventative health strategies and patient management, particularly in populations where liver health is a concern.

Our study also has limitations. The first notable limitation of our study is the absence of detailed data categorizing specific liver-related deaths within the NHANES dataset. This limitation precludes a focused analysis on the individual contributions of liver diseases such as cirrhosis, liver cancer, and acute hepatitis to overall mortality. While liver-related deaths constitute a relatively small fraction (1%) of all-cause mortality in this dataset [47], the lack of specific categorization limits our ability to precisely delineate the impact of liver function biomarkers on mortality attributed to these specific liver conditions. Second, the predictive performance of liver function biomarkers for mortality has not been externally validated, although we have performed internal validation and applied cross-validation measures to ensure robustness. Third, this type of population-based study lacks insights for understanding the underlying mechanisms. The between-study heterogeneity is therefore barely explained and is mainly ascribed to the variations among different populations. Finally, data for all variables were available exclusively at baseline, so we could not use time-varying variables to capture possible changes in the results over time. However, we initially excluded the participants who had an obviously abnormal level of liver function biomarkers to avoid the potential biases introduced by transient increases or decreases. The baseline measurement of these biomarkers might serve as a surrogate for the long-term serum level.

In summary, our study suggests positive independent associations of baseline circulating levels of GGT and ALP with all-cause and cause-specific mortality risk. A negative association was observed between ALB levels and risk of mortality. Any association of ALT, AST, and TB with mortality risk is comparatively moderate and nonlinear and requires further confirmation. The overall findings highlight the pivotal role of the liver in life processes and

suggest that circulating liver function biomarkers may be informative of survival in general populations and could serve as screening tools to identify individuals at high risk of death.

Abbreviations

NHANES	National Health and Nutrition Examination Survey
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GGT	Gamma-glutamyl transferase
ALP	Alkaline phosphatase
TB	Total bilirubin
ALB	Albumin
HR	Hazard ratio
LFRS	Liver function risk score
AUROC	Area under receiver operating characteristic curve

Supplementary Information

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Supplementary Material 1.

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Not applicable

Authors' contributions
SHL and LHS conceived and designed the study. SHL and HPD developed the methodology. SHL, LHS, HPD and GBL analyzed and interpreted the data. SHL is responsible for first manuscript draft, revised and/or reviewed by HPD, GBL and LHS. All authors contributed to the article and approved the submitted version.

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Data availability
The datasets supporting the conclusions of this article are included within the article.

Declarations

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Not applicable.

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Competing interests
The authors declare no competing interests.

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References

1. Watt MJ, Miotto PM, De Nardo W, et al. The Liver as an endocrine organ-linking NAFLD and insulin resistance. Endocr Rev. 2019;40:1367–93.
2. Marques PE, Oliveira AG, Chang L, et al. Understanding liver immunology using intravital microscopy. J Hepatol. 2015;63:733–42.
3. Loria P, Carulli L, Bertolotti M, et al. Endocrine and liver interaction: the role of endocrine pathways in NASH. Nat Rev Gastroenterol Hepatol. 2009;6:236–47.
4. Hampel PJ, Chaffee KG, King RL, et al. Liver dysfunction in chronic lymphocytic leukemia: prevalence, outcomes, and pathological findings. Am J Hematol. 2017;92:1362–9.
5. Freire Jorge P, Wieringa N, de Felice E, et al. The association of early combined lactate and glucose levels with subsequent renal and liver dysfunction and hospital mortality in critically ill patients. Crit Care. 2017;21:218.
6. Zhuang X, Cui AM, Wang Q, et al. Liver dysfunction during pregnancy and its association of with preterm birth in china: a prospective cohort study. EBioMedicine. 2017;26:152–6.
7. van Slambrouck CM, Salem F, Meehan SM, et al. Bile cast nephropathy is a common pathologic finding for kidney injury associated with severe liver dysfunction. Kidney Int. 2013;84:192–7.
8. Putz-Bankuti C, Pilz S, Stojakovic T, et al. Association of 25-hydroxyvitamin D levels with liver dysfunction and mortality in chronic liver disease. Liver Int. 2012;32:845–51.
9. Armstrong MJ, Adams LA, Canbay A, et al. Extrahepatic complications of nonalcoholic fatty liver disease. Hepatology. 2014;59:1174–97.
10. Calori G, Lattuada G, Ragogna F, et al. Fatty liver index and mortality: the Cremona study in the 15th year of follow-up. Hepatology. 2011;54:145–52.
11. Kunutsor SK, Apekey TA, Seddoh D, et al. Liver enzymes and risk of all-cause mortality in general populations: a systematic review and meta-analysis. Int J Epidemiol. 2014;43:187–201.
12. Koehler EM, Sanna D, Hansen BE, et al. Serum liver enzymes are associated with all-cause mortality in an elderly population. Liver Int. 2014;34:296–304.
13. Erlinger S, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. Gastroenterology. 2014;146:1625–38.
14. Montero D, Diaz-Canestro C, Oberholzer L, et al. The role of blood volume in cardiac dysfunction and reduced exercise tolerance in patients with diabetes. Lancet Diabetes Endocrinol. 2019;7:807–16.
15. Xie K, Chen CH, Tsai SP, et al. Loss of life expectancy by 10 years or more from elevated aspartate aminotransferase: finding aspartate aminotransferase a better mortality predictor for all-cause and liver-related than alanine aminotransferase. Am J Gastroenterol. 2019;114:1478–87.
16. Kunutsor SK, Apekey TA, Walley J. Liver aminotransferases and risk of incident type 2 diabetes: a systematic review and meta-analysis. Am J Epidemiol. 2013;178:159–71.
17. Ruhl CE, Everhart JE. The association of low serum alanine aminotransferase activity with mortality in the US population. Am J Epidemiol. 2013;178:1702–11.
18. Ruttmann E, Brant LJ, Concin H, et al. Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. Circulation. 2005;112:2130–7.
19. Hernaez R, Yeh HC, Lazo M, et al. Elevated ALT and GGT predict all-cause mortality and hepatocellular carcinoma in Taiwanese male: a case-cohort study. Hepatol Int. 2013;7:1040–9.
20. Centers for Disease Control and Prevention. About the National Health and Nutrition Examination Survey. https://www.cdc.gov/nchs/nhanes/about_nhanes.htm. Accessed at Oct 2, 2019.
21. Chen C, Ye Y, Zhang Y, et al. Weight change across adulthood in relation to all cause and cause specific mortality: prospective cohort study. BMJ. 2019;367:l5584.
22. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol. 2003;98:960–7.
23. National Center for Health Statistics. 2015 Public-Use Linked Mortality Files. 2019. https://www.cdc.gov/nchs/data-linkage/mortality-public.htm#. Accessed on Oct 1, 2019.
24. Unalp-Arida A, Ruhl CE. Noninvasive fatty liver markers predict liver disease mortality in the U.S. population. Hepatology. 2016;63:1170–83.

25. Meira-Machado L, Cadarso-Suarez C, Gude F, et al. smoothHR: an R package for pointwise nonparametric estimation of hazard ratio curves of continuous predictors. Comput Math Methods Med. 2013;2013:745742.
26. Kim HC, Nam CM, Jee SH, et al. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. BMJ. 2004;328:983.
27. Tonelli M, Curhan G, Pfeffer M, et al. Relation between alkaline phosphatase, serum phosphate, and all-cause or cardiovascular mortality. Circulation. 2009;120:1784–92.
28. Breitling LP, Claessen H, Drath C, et al. Gamma-glutamyltransferase, general and cause-specific mortality in 19,000 construction workers followed over 20 years. J Hepatol. 2011;55:594–601.
29. Ruhl CE, Everhart JE. Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. Gastroenterology. 2009;136:477-85.e11.
30. Nakamura K, Okamura T, Kanda H, et al. The value of combining serum alanine aminotransferase levels and body mass index to predict mortality and medical costs: a 10-year follow-up study of National Health Insurance in Shiga. Japan J Epidemiol. 2006;16:15–20.
31. Williams KH, Sullivan DR, Nicholson GC, et al. Opposite associations between alanine aminotransferase and gamma-glutamyl transferase levels and all-cause mortality in type 2 diabetes: Analysis of the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. Metabolism. 2016;65:783–93.
32. Lee TH, Kim WR, Benson JT, et al. Serum aminotransferase activity and mortality risk in a United States community. Hepatology. 2008;47:880–7.
33. Kengne AP, Czernichow S, Stamatakis E, et al. Gamma-glutamyltransferase and risk of cardiovascular disease mortality in people with and without diabetes: pooling of three British Health Surveys. J Hepatol. 2012;57:1083–9.
34. Yi SW, Lee SH, Hwang HJ, et al. Gamma-glutamyltransferase and cardiovascular mortality in Korean adults: a cohort study. Atherosclerosis. 2017;265:102–9.
35. Kunutsor SK, Khan H, Laukkanen JA. gamma-Glutamyltransferase and risk of sudden cardiac death in middle-aged Finnish men: a new prospective cohort study. J Am Heart Assoc. 2016;5:e002858.
36. Kabootari M, Raee MR, Akbarpour S, et al. Serum alkaline phosphatase and the risk of coronary heart disease, stroke and all-cause mortality: Tehran Lipid and Glucose Study. BMJ Open. 2018;8:e023735.
37. Wannamethee SG, Sattar N, Papcosta O, et al. Alkaline phosphatase, serum phosphate, and incident cardiovascular disease and total mortality in older men. Arterioscler Thromb Vasc Biol. 2013;33:1070–6.
38. Park JB, Kang DY, Yang HM, et al. Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent. Eur Heart J. 2013;34:920–31.
39. McCallum L, Panniyammakal J, Hastie CE, et al. Longitudinal Blood Pressure Control, Long-Term Mortality, and Predictive Utility of Serum Liver Enzymes and Bilirubin in Hypertensive Patients. Hypertension. 2015;66:37–43.
40. Horsfall LJ, Rait G, Walters K, et al. Serum bilirubin and risk of respiratory disease and death. JAMA. 2011;305:691–7.
41. Su HH, Kao CM, Lin YC, et al. Relationship between serum total bilirubin levels and mortality in uremia patients undergoing long-term hemodialysis: a nationwide cohort study. Atherosclerosis. 2017;265:155–61.
42. Corti MC, Guralnik JM, Salive ME, et al. Serum albumin level and physical disability as predictors of mortality in older persons. JAMA. 1994;272:1036–42.
43. Djousse L, Rothman KJ, Cupples LA, et al. Serum albumin and risk of myocardial infarction and all-cause mortality in the Framingham Offspring Study. Circulation. 2002;106:2919–24.
44. Wu CY, Hu HY, Huang N, et al. Albumin levels and cause-specific mortality in community-dwelling older adults. Prev Med. 2018;112:145–51.
45. Scherzer R, Heymsfield SB, Rimland D, et al. Association of serum albumin and aspartate transaminase with 5-year all-cause mortality in HIV/hepatitis C virus coinfection and HIV monoinfection. AIDS. 2017;31:71–9.
46. Sudfeld CR, Isanaka S, Aboud S, et al. Association of serum albumin concentration with mortality, morbidity, CD4 T-cell reconstitution among tanzanians initiating antiretroviral therapy. J Infect Dis. 2013;207:1370–8.
47. Unalp-Arida A, Ruhl CE. Patatin-Like Phospholipase Domain-Containing Protein 3 I148M and Liver Fat and Fibrosis Scores Predict Liver Disease Mortality in the U.S. Population Hepatology. 2020;71:820–34.

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