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Serum liver enzymes are associated with all-cause mortality in an elderly population

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alkaline phosphatase – aminotransferases – elderly – epidemiology – gamma-glutamyltransferase – liver enzymes – mortality

Abbreviations
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CVD, cardiovascular diseases; GGT, Gamma-glutamyltransferase.

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Abstract

Background & Aims: Little is known about the association of serum liver enzymes with long-term outcome in the elderly. We sought to clarify the association of serum gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) with all-cause and cause-specific mortality in an elderly population. *Methods:* This study was embedded in the Rotterdam Study, a large population-based cohort of persons aged 55 years or older. Cox-regression analyses were performed to examine the association of baseline serum GGT, ALP, and aminotransferase levels with mortality, adjusted for age, sex, education, smoking status, alcohol intake, hypertension, diabetes mellitus, body mass index and total cholesterol levels. Liver enzyme levels were categorized according to sample percentiles; levels <25th percentile were taken as a reference. *Results:* During a follow-up of up to 19.5 years, 2997 of 5186(57.8%) participants died: 672 participants died of causes related to cardiovascular diseases (CVD) and 703 participants died of cancer. All serum liver enzymes were associated with all-cause mortality (all $P < 0.001$). Moreover, GGT was associated with increased CVD mortality ($P < 0.001$), and ALP and AST with increased cancer-related mortality ($P = 0.03$ and $P = 0.005$ respectively). Participants with GGT and ALP in the top 5% had the highest risk for all-cause mortality (HR1.55; 95%CI 1.30–1.85 and HR1.49; 95%CI 1.25–1.78 respectively). AST and ALT <25th percentile were also associated with a higher risk of all-cause mortality. *Conclusions:* All serum liver enzymes were positively associated with long-term mortality in this elderly population. Why participants with low ALT and AST levels have higher risk of mortality remains to be elucidated.

Serum gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are commonly used markers of liver injury. ALT is predominantly found in the liver, whereas GGT and AST are expressed in multiple other tissues, including heart, skeletal muscle, kidneys and brain (1). ALP is also present at many locations, but is primarily expressed in liver and bone (2). 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 (3, 4). ALP catalyses the hydrolysis of organic phosphate esters and AST and ALT catalyse the transfer of amino groups to form the hepatic metabolites pyruvate and oxaloacetate respectively.

Elevations of serum liver chemistry tests are reported in up to 24.5% of individuals in Western community- or population-based studies (5–7). The majority of liver test abnormalities may be attributed to the presence of alcoholic and nonalcoholic fatty liver disease (8). Additionally, elevations of ALP are associated with cholestatic

liver diseases and bone diseases. However, a considerable proportion of liver enzyme elevations remains unexplained (9).

Currently, there is increasing interest for the role of liver enzymes as independent predictors of non liver-related morbidity and mortality. Elevations of GGT, ALT and AST have been associated with a higher incidence of cancer and CVD, and higher risk of overall and CVD-related mortality (6, 10). To date, two studies examined the association between elevation of ALP and mortality (11, 12). However, only few studies investigated associations of the whole range of liver biochemistries with mortality (13–15). Moreover, the aging population is the greatest consumer of healthcare, and given that the majority of studies included mainly younger adults, the role of these inexpensive blood tests as predictors of mortality in the elderly remains to be determined.

The aim of this study was to investigate the association of serum GGT, ALP, ALT and AST levels with all-cause and cause-specific mortality in an elderly population.

Materials and methods

Study population

The current study was embedded in the Rotterdam Study, a large prospective population-based cohort study, with the objective of examining the occurrence and risk factors of chronic diseases in the elderly. The study design and objectives have been described in detail previously (16). From 1990 to 1993, all inhabitants of the Ommoord district of Rotterdam, the Netherlands, aged 55 years or older, were invited for participation. Of 10 275 invitees, 7983 (78%) agreed to participate. During baseline examinations, participants had an extensive at-home interview and subsequently visited the research centre for a clinical examination and blood collection. The medical ethics committee at Erasmus University of Rotterdam approved the study, and written informed consent was obtained from all participants.

Serum liver chemistry tests

Non-fasting and fasting blood samples were collected by venapuncture, and immediately frozen (−20°C). Serum GGT, ALP, ALT and AST levels were determined within two weeks using a Merck Diagnostica kit (Merck, Whitehouse Station, NJ, USA) on an Elan Autoanalyzer (Merck). All liver biochemistry measurements were obtained in the laboratory of the Department of Epidemiology, Erasmus University Medical Center. Non-fasting samples were considered acceptable, for normal food intake does not greatly affect serum liver enzyme levels (17, 18). Liver enzyme tests were performed only until December 31, 1992, when they were stopped because of financial constraints. According to local cut-offs, elevation of GGT was defined as >34 U/L for women and >49 U/L for men, and elevation of ALP was defined as >97 U/L for women and >114 U/L for men. Elevation of ALT was defined as >30 U/L for women and >40 U/L for men, and elevation of AST was defined as >30 U/L for women and >36 U/L for men.

Assessment of mortality

Information on vital status was obtained on a regular basis from the central registry of the Municipality of Rotterdam, from collaborating general practitioners and by obtaining information during follow-up rounds. The Central Registry of Genealogy of the Netherlands was consulted for data on participants with missing information on vital status. Two research physicians independently classified events according to the International Classification of Diseases, 10th revision (ICD-10) (19). We used the underlying cause of death, which is the disease or injury, which initiated the train of events leading directly to death. In case of disagreement, consensus was reached in a separate session. A medical expert in the field reviewed all coded events for a final classification. The following ICD codes were considered for cause-specific mortality: (i) *CVD*: I20-25, I42, I46, I50, I 63, I66, I67.2, I67.8, I69.3, I70, I70.9, I74; (ii) *cancers*: all C-codes. Liver diseases had ICD codes K70, K72, or K73.

Participants were followed up until death or January 1st, 2009, whichever came first.

Assessment of covariables

In the interview preceding the clinical examination, data were obtained concerning demographics, medical history, comorbid conditions, smoking behaviour and drug use. Additionally, detailed information on drug prescriptions was dispensed from local pharmacies. Weekly alcohol consumption was obtained by means of a validated food-frequency questionnaire and recalculated into grams per day (20). Anthropometric measurements were performed at the research centre. BMI was calculated as weight (kg)/ height (m^2). Waist and hip circumference were measured in centimetres. The average of two blood pressure measurements, obtained at a single visit in sitting position after 5 min rest, was used for analysis. Hypertension was defined as blood pressure ≥140/90 mmHg or drug treatment for elevated blood pressure. Diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dl (7.0 mmol/L) or non-fasting plasma glucose ≥200 mg/dl (11.1 mmol/L) or drug treatment for elevated blood glucose. Cholesterol levels were defined as desirable (<200 mg/dl or <5.1 mmol/L), borderline high (200–240 mg/dl or 5.1–6.1 mmol/L) or high (≥240 mg/dl or ≥6.2 mmol/L), according to Adult Treatment Panel (ATP) III criteria. Highest attained educational level was grouped according to the

Dutch Standard Classification of Education and split into three categories: lower (primary education only), intermediate (lower vocational or general education) or higher education (intermediate or higher vocational or general education, university) (21). Presence of CVD at baseline was defined as prior myocardial infarction, stroke, coronary artery bypass graft or percutaneous transluminal coronary angioplasty. This information was initially self-reported and later confirmed using physician or hospital records or an electrocardiogram showing characteristics of prior myocardial infarction.

Statistical analyses

Baseline analyses were performed using descriptive statistics. Chi-square tests and Student's *t*-tests (means) or Wilcoxon rank sum tests (medians) were used to assess the significance of differences in distributions of categorical data and continuous data respectively. Serum GGT, ALP, ALT and AST levels were categorized into percentiles for men and women separately (<25th, 25th–<50th, 50–<75th, 75th–<95th and ≥95th percentile). To reduce bias, missing values on covariables were imputed by multiple imputation applying the Markov chain Monte Carlo method. All variables, covariables and outcome variables were used to generate imputations of missing values and 10 datasets were imputed (MI procedure and MI analyse procedure) (22, 23). The Cox proportional hazard model was used to evaluate associations between serum GGT, ALP, ALT and AST levels and all-cause or cause-specific mortality. After adjustment for age and sex, further models were adjusted for education, smoking status, alcohol intake, hypertension, diabetes mellitus, BMI and total cholesterol levels. Age was modelled as a continuous variable. Covariables like smoking status and hypertension were replaced by continuous variables (packyears and both systolic and diastolic blood pressure respectively) in additional analyses to check for consistency of the model. In addition, BMI was replaced by waist circumference, for there is collinearity between these two covariables. Liver enzyme levels <25th percentile were taken as a reference. Models were tested for interaction with age, sex, BMI and alcohol intake. To adjust for multiple testing, level for interaction was set to 0.01. Additional sensitivity analyses were performed excluding (i) participants with cardiovascular disease at baseline, (ii) former drinkers, (iii) participants with >8 times the upper limit of normal of serum liver biochemistries (for these participants may have had acute hepatitis) and (iv) participants that died during the first three years of follow-up (because these participants may have had underlying terminal illness). Furthermore, covariables that might be associated with ALP levels, including haemoglobin levels and serum phosphate levels, were added to models of ALP. A cubic spline regression was performed to illustrate the association of the continuous liver test measurements with all-cause mortality. Knots were placed at the 5th, 27.5th, 50th, 72.5th and 95th percentiles; graphs were plotted using the SAS LGTPHCURV9 Macro (24). SAS version 9.2 (SAS Institute Inc, Cary, NC, USA) was used for all statistical analyses. A *P*-value of 0.05 was considered statistically significant.

Results

Baseline characteristics

Serum GGT, ALP, ALT and AST levels were tested for 5186 participants. Baseline characteristics are illustrated in Table 1. Mean age of participants was 70.3 ± 9.1 years (range: 55–99 years) and 61.6% of the population was female. The vast majority of the population was of Caucasian ethnicity. According to local cut-offs of liver enzyme levels, 15.1% of the population had elevated GGT, 13.8% had elevated levels of ALP, 5.2% had elevated levels of ALT and 4.2% had elevated levels of AST. Values for percentiles of each liver enzyme in the study population are shown in Table 2.

Table 1. Baseline characteristics of the study population (n = 5186)

Characteristic	
Age (years)	70.3 ± 9.1
Female	61.6
Caucasian	91.7
Education, low	56.3
Smoking	
Never	34.1
Former	39.5
Current	23.0
BMI (kg/m^2)	26.3 ± 3.8
Diabetes Mellitus	8.1
Hypertension	56.8
Total Cholesterol	
<200 mg/dl	10.7
200–239 mg/dl	25.5
≥240 mg/dl	63.8
Alcohol intake	
Non-drinker	36.1
≤10 g/day	29.4
>10 ≤ 30 g/day	24.8
>30 g/day	9.7
Cardiovascular disease	33.6
Inorganic phosphate (mg/dl)	3.7 (3.3–4.1)
Haemoglobin (g/dl)	14.1 (13.2–15.0)
GGT (U/L)	23 (17–32)
ALP (U/L)	76 (63–91)
ALT (U/L)	16 (12–20)
AST (U/L)	19 (16–22)

Values are mean ± SD, percentage, or median (interquartile range).
BMI, body mass index; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 2. Percentile values of serum GGT, ALP, ALT and AST levels for men and women in the study population

	25th percentile	50th percentile	75th percentile	95th percentile
GGT (U/L)				
Male	20	26	37	79
Female	16	21	28	58
ALP (U/L)				
Male	61	73	89	119
Female	64	77	92	124
ALT (U/L)				
Male	13	17	22	35
Female	12	15	19	33
AST (U/L)				
Male	17	20	33	31
Female	16	19	22	30

GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Association of liver enzymes with established risk factors

In univariable analysis, higher serum GGT levels were associated with lower age, male sex, presence of diabetes mellitus, hypertension, and current smoking, and higher BMI, cholesterol levels and alcohol intake (Table S1). Higher serum ALP levels were associated with higher age, female gender, presence of diabetes mellitus, current smoking and hypertension, lower cholesterol levels and lower alcohol intake. Serum ALT levels were inversely associated with age and positively associated with female sex, current smoking, presence of diabetes mellitus, hypertension and alcohol intake. Finally, AST levels were inversely associated with age, and positively associated with male gender, hypertension and alcohol intake.

Mortality in the study population

Median follow-up was 14.0 years [interquartile range (IQR) 6.8–16.3 years, with a maximum of 19.5 years]. During follow-up, 2997 (57.8%) participants died; 672 participants died of cardiovascular-related causes (22.4% of total deaths) and 703 participants died of cancer (23.4% of total deaths). Stroke was the cause of death in 9.9% of total deaths and ischaemic heart disease in 5.3% of total deaths. Eight per cent of total deaths were from neurodegenerative diseases and 6.5% from diseases of the respiratory system. Five participants died from liver diseases (4 deaths were alcohol-related) and 7 participants died from primary liver cancer (hepatocellular carcinoma: 3; cholangiocarcinoma: 4).

Association of cholestatic liver enzymes with all-cause and cause-specific mortality

Gamma-glutamyltransferase was positively associated with all-cause mortality ($P < 0.001$) and CVD-related mortality ($P < 0.001$) after adjustment for age, sex and all other potential confounders, if studied continuously (Table S2). Figure 1A illustrates adjusted hazard ratios (HR) and 95% confidence intervals (CI) for GGT percentiles. The HR for participants with GGT ≥95th percentile (58 U/L for women and 79 U/L for men) was 1.62 (95% CI 1.36–1.92) after adjustment for age and sex, and 1.55 (95% CI 1.30–1.85) after adjustment for all other potential confounders. When GGT was fitted as a spline with median GGT as a reference, an almost linear relationship was observed (Fig. 1B).

After adjustment for all potential confounders, ALP was positively associated with all-cause mortality ($P < 0.001$) and cancer-related mortality ($P = 0.03$) and showed a trend for association with CVD mortality ($P = 0.08$) (Fig. 1C, Table S2). Adjusted HR for all-cause mortality for participants with ALP ≥95th percentile (124 U/L for women and 119 U/L for men) was 1.50 (95% CI 1.25–1.79). Findings remained consistent when haemoglobin levels and serum phosphate levels were added in the model and when analyses were restricted to participants with normal haemoglobin levels. Cubic spline regression for ALP is illustrated in Fig. 1D.

Association of aminotransferases with all-cause and cause-specific mortality

Alanine aminotransferase was positively associated with all-cause mortality ($P < 0.001$; Fig. 2A, Table S3). Highest risks were observed for participants with ALT <25th percentile (<12 U/L for women and <13 U/L for men; HR 1 (*ref*)) and ALT ≥95th percentile (33 U/L for women and 35 U/L for men; HR 0.92, 95% CI 0.76–1.11). This J-shaped relationship is also illustrated in Fig. 2B, where ALT is fitted as a spline with the median as a reference.

Aspartate aminotransferase was positively associated with all-cause mortality ($P < 0.001$) and cancer-related mortality ($P = 0.005$; Fig. 2C, Table S3). Participants with AST ≥95th percentile (30 U/L for women and 31 U/L for men) had a HR of 1.12 (95% CI 0.95–1.32). Similar to ALT, a J-shaped curve was observed (Fig. 2D). AST showed an interaction with age. AST was not associated with all-cause mortality for age >76.8 years.

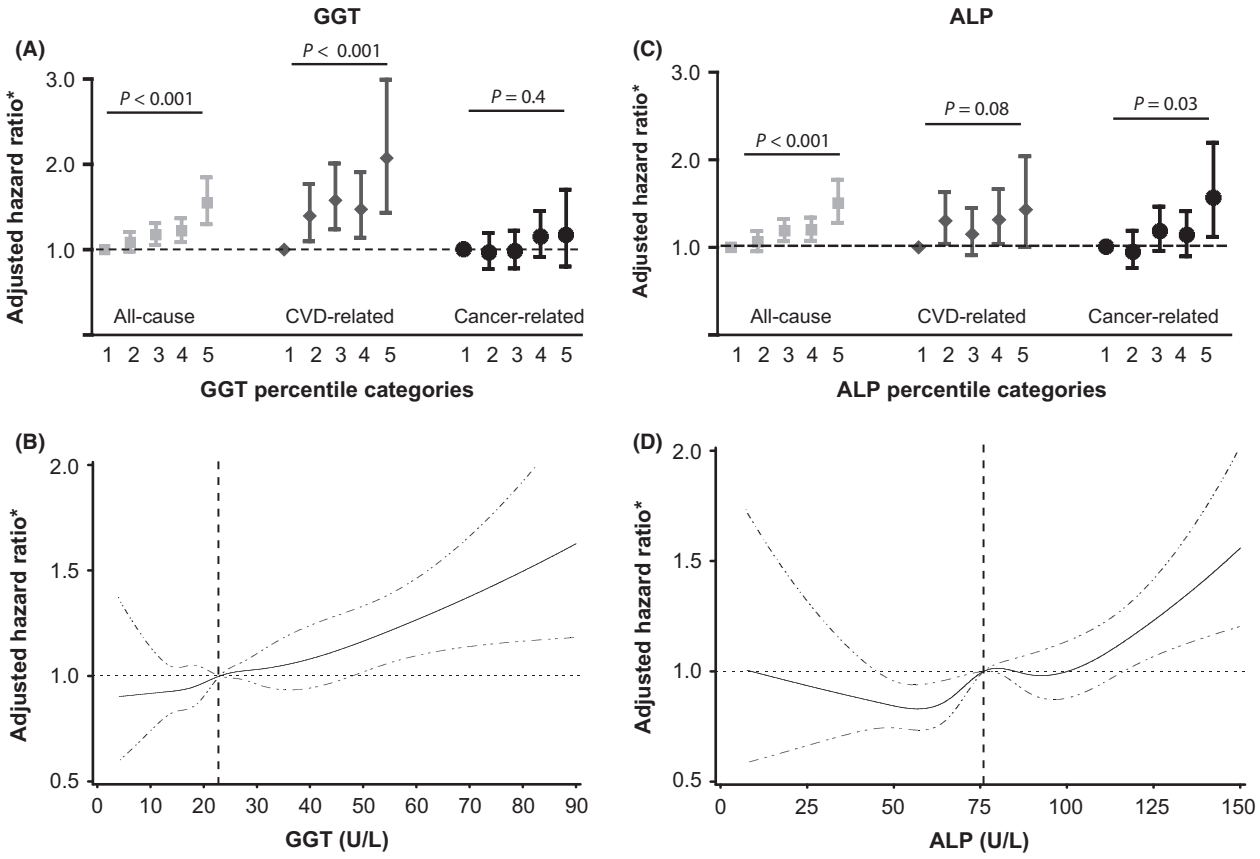

Fig. 1. Association of cholestatic liver enzymes with all-cause and cause-specific mortality**.** (A) Adjusted Hazard Ratios (HRs) for mortality by percentiles of serum GGT. Reference: <25th percentile. HRs are adjusted for age, sex, education, smoking status, alcohol intake, hypertension, diabetes mellitus, BMI and total cholesterol levels. Percentiles are illustrated as follows: category 1: <25th percentile; category 2: 25th–<50th percentile; category 3: 50th–<75th percentile; category 4: 75th–<95th percentile; category 5: ≥95th percentile. (B) Association of all-cause mortality with serum GGT levels, illustrated as a spline with the median as a reference. (C) Adjusted HRs for mortality by percentiles of serum ALP. (D) Association of all-cause mortality with serum ALP levels, illustrated as a spline with the median as a reference.

The model including AST and all potential confounders had lower -2 log likelihood than models including GGT, ALP and ALT (37131 vs. 37138, 37136 and 37145 respectively), suggesting a stronger fit of the model.

In the Cox-regression analyses, no interaction was found between any of the liver biochemistries and sex, BMI or alcohol consumption. Age was not an effect modifier for the association of GGT, ALP and ALT with mortality.

Sensitivity analyses were performed regarding all liver tests and results remained consistent. Only when analyses were restricted to participants without a baseline history of CVD, no association was observed between GGT and CVD mortality. However, the pattern did almost not diverge from the original pattern. Adjustment for waist circumference instead of BMI did not change results, and adjustment for continuous variables for smoking status (pack years) and hypertension (diastolic and systolic blood pressure) did not diverge the model.

Discussion

In this large population-based cohort study of participants older than 55 years, we demonstrated that serum GGT, ALP, ALT and AST levels are associated with all-cause mortality. Secondly, GGT was associated with CVD-related mortality, and ALP and AST with cancer-related mortality.

A unique aspect of this study is its well-characterized design, long-term follow-up and large number of events. Moreover, this study was population-based, has an excellent participation rate of 78%, and mortality was continuously and accurately monitored through linkage with records of GPs, hospitals and nursing homes.

We analysed our data using percentiles of serum liver tests, for we were interested in studying the association of the whole range of serum GGT, ALP and aminotransferase levels with mortality. Moreover, no upper limits of normal (ULN) have been defined for elderly participants, as blood donor studies mainly include younger

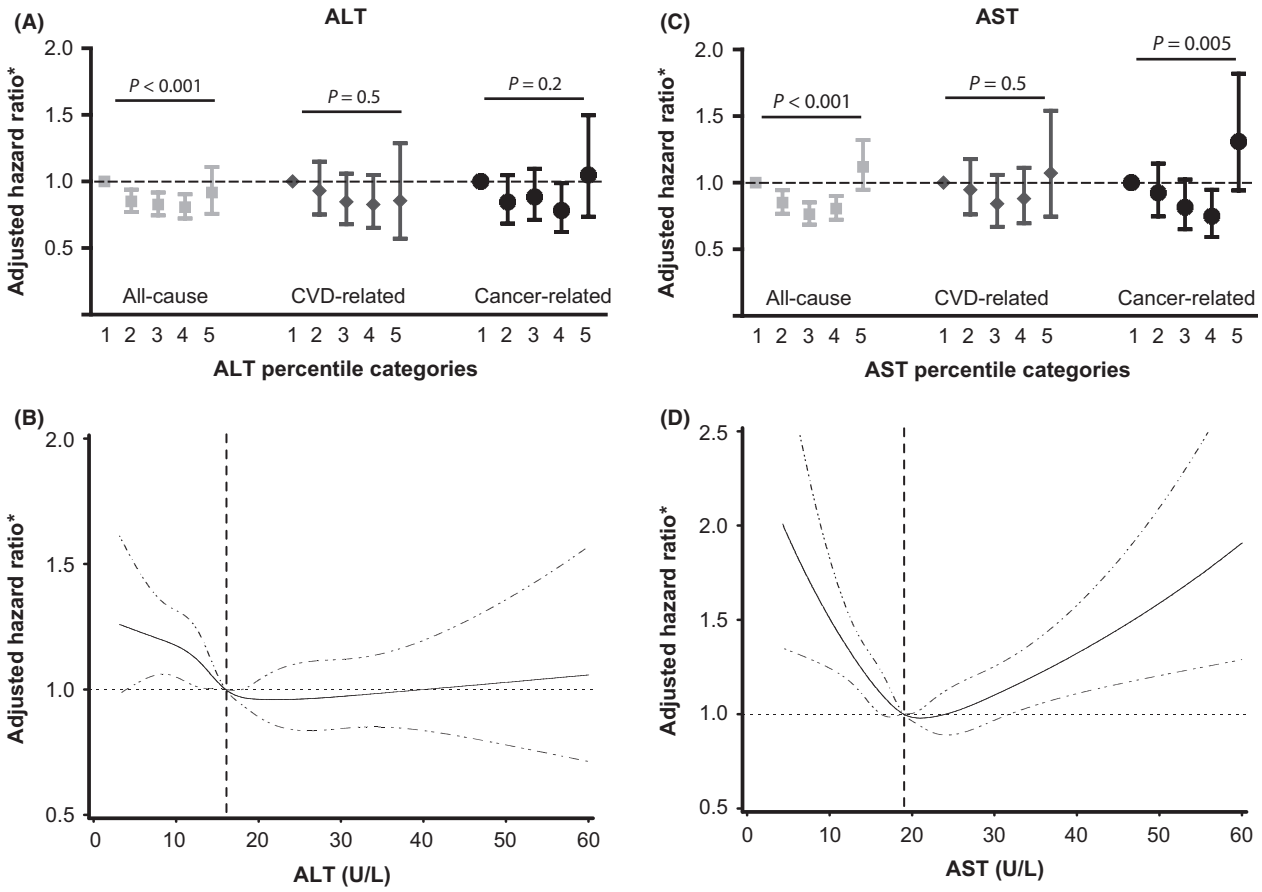

Fig. 2. Association of aminotransferases with all-cause and cause-specific mortality**.** (A) Adjusted HRs for mortality by percentiles of serum ALT. Reference: <25th percentile. HRs are adjusted for age, sex, education, smoking status, alcohol intake, hypertension, diabetes mellitus, BMI and total cholesterol levels. Percentiles are illustrated as follows: category 1: <25th percentile; category 2: 25th–<50th percentile; category 3: 50th–<75th percentile; category 4: 75th–<95th percentile; category 5: ≥95th percentile. (B) Association of all-cause mortality with serum ALT levels, illustrated as a spline with the median as a reference. (C) Adjusted HRs for mortality by percentiles of serum AST. (D) Association of all-cause mortality with serum AST levels, illustrated as a spline with the median as a reference.

adults and ULN of ALT decreases with age (25–27). When we analysed the association between serum liver biochemistries by spline regression, similar outcomes were obtained.

For GGT, our results are consistent with previous studies demonstrating increased overall and CVD mortality with increasing GGT levels in various, younger populations (6, 10, 14, 28, 29). Few studies observed an interaction with age. Lee *et al.* showed that the relationship between elevated GGT and increased CVD mortality was only present in subjects younger than 55 years and not in elderly subjects (29). Our study mainly consisted of elderly participants and we did not find interaction between age and GGT. Although GGT levels were not significantly associated with CVD mortality after excluding participants with CVD at baseline, these results should be interpreted carefully, as almost one-third of participants were excluded for these sensitivity analyses and results showed an almost similar pattern. Given our results and evidence from previous studies, GGT appears to be an important indicator of overall health and CVD health, even in the elderly. It has been suggested that GGT levels may capture processes relevant to atherogenesis or may play a role in the development of oxidative stress (30).

Only two studies investigated the relationship between elevation of ALP and mortality in population samples (11, 12). Tonelli *et al.* demonstrated an independent relationship of ALP with all-cause and CVD mortality in adult subjects from the general US population (11). In the present study, serum ALP levels are associated with all-cause and cancer-related mortality and showed a trend for association with CVD mortality. To date, little is known about the physiological functioning of ALP. ALP may be involved in vascular calcification processes outside the context of renal failure, which may explain the observed trend for association between ALP and CVD mortality (11, 31).

In contrast to the strong body of evidence for GGT, recent studies of community or population-based samples investigating the relationship of ALT and AST with mortality are less congruent. A large Korean study confirmed the association of the whole range of ALT with increased mortality in men, but not in women (13). However, Asian populations have a different distribution of mortality causes from that of Western populations (32, 33). Secondly, using data from NHANES III, Ong *et al.* found that elevated serum aminotransferases in the absence of significant alcohol intake and other liver diseases was also significantly associated with higher mortality risk (34). An analysis with the same dataset using slightly different selection criteria and a different statistical calculation of variance did not demonstrate a statistically significant association (6). We demonstrated that aminotransferases are associated with all-cause mortality in an elderly population. However, for AST, the association with all-cause mortality was not significant above age 76.

In the present study, AST showed stronger association with all-cause mortality than ALT. In part, this may be because of the association of AST with cancer-related mortality. Moreover, from a biological perspective, ALT is abundantly expressed in liver tissue, whereas AST is expressed in multiple other tissues. Given the small number of events of specific causes of CVD or cancer, leading to large confidence intervals, we were unable to determine the association with cause-specific mortality in more detail.

Remarkably, GGT and ALP levels showed an almost linear relationship with mortality risk, whereas ALT and AST showed a J-shaped relationship. Similar observations have been published previously and it has been suggested that overall or hepatic frailty, or hepatic aging may underlie these observations (35). Hepatic aging has been associated with greater oxidative stress and hepatic cell apoptosis in rat livers (36). Furthermore, in early studies on aminotransferases, it was demonstrated that low transaminase levels may be caused by pyridoxine deficiency (37). In turn, pyridoxine deficiency is generally the result of decreased intake of vitamin B6. In our analyses, we were not able to adjust for pyridoxine levels or intake of vitamin B6, but did correct for BMI. Furthermore, there was no interaction of BMI with any of the other covariables. Nevertheless, low BMI may also reflect low nutritional intake and is associated with higher mortality (38). Additional research is required to further elucidate the mechanism of these observations.

To date, individuals over 55 years of age constitute the fastest growing segment in Western populations and greatest consumers of healthcare. Liver chemistry tests may represent valuable markers of long-term outcome in this segment of the population. Although screening for these tests may aid clinicians to identify conditions that may lead to significant morbidity and mortality in elderly persons, the enormous burden for healthcare practice in terms of cost-effectiveness and provision of services and healthcare providers should also be considered.

A limitation of this study is the inability of excluding subjects with viral hepatitis at baseline. However, the prevalence of hepatitis B and C in the Dutch population is estimated to be very low (39, 40). We performed additional sensitivity analyses excluding participants with possible acute hepatitis, and did not obtain significant differences on results. Moreover, in the present study, we performed analyses using only a single measurement of GGT, ALP, ALT or AST. Liver enzyme examinations were stopped before all participants had visited the research centre. Because participants were invited in a semi-random order, by postal code, it is unlikely that this affected our results. Finally, our results may not be generalized to other ethnic populations as the vast majority of this study population was of Caucasian ethnicity.

In summary, the current study sought to clarify the association between serum GGT, ALP, ALT and AST levels with long-term mortality in a large prospective population-based study of mainly Caucasian elderly. All liver enzymes were positively associated with all-cause mortality. Therefore, these tests may represent useful indicators of longevity in the elderly. Moreover, in this elderly population, we found an association between low levels of ALT and AST and increased mortality of all causes. Although clinicians are generally concerned about significant increases in transaminases, moderate increases or very low levels may also be clinically relevant.

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Conflict of interest: The authors do not have any disclosures to report.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Association between liver enzymes and covariables in univariate analyses. Illustrated are correlation coefficients and standard errors.

Table S2. Association of cholestatic liver enzymes with all-cause and cause-specific mortality. Percentiles are shown in categories: (i) <25th percentile; (ii) 25th–<50th percentile; (iii) 50th–<75th percentile; (iv) 75th–<95th percentile; (v) ≥95th percentile.

Table S3. Association of aminotransferases with all-cause and cause-specific mortality. Percentiles are shown in categories: (1) <25th percentile; (2) 25th–<50th percentile; (3) 50th–<75th percentile; (4) 75th–<95th percentile; (5) ≥95th percentile.