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Elevated liver enzymes and mortality in older individuals: a prospective cohort study

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ABSTRACT

Aim: To determine the excess risk of all-cause and cardiovascular mortality in older people with elevated liver enzymes (alanine transaminase, ALT and gamma glutamyltransferase, GGT).

Methods: We utilized data from a large, prospective, population based study of 2,061 people aged 50-99 years with linkage to a national death registry. Participants were categorized as having elevated liver enzymes using standard thresholds (for males, GGT >51 and ALT >40 IU/L, and GGT >33 and ALT >31 IU/L for females). Adjusted Cox proportional hazards models assessed the association of elevated liver enzymes and mortality with long duration follow up.

Results: Over a median follow up of 10 years (20,145 person years), 701 people died, including 203 (34%) from cardiovascular disease. Cox regression models adjusted for sex, age, smoking and alcohol intake indicated that people with elevated liver enzymes had an increased risk of all-cause mortality that was modified by age (test for interaction p=0.01). Age-stratified analyses demonstrated no increased risk at younger ages (age ≤ 59 years, H.R. 0.46 95% confidence interval 0.06-3.49), but increased risk with age; age 60-69, H.R. 1.05 (0.53-2.07), age 70-79 years, H.R. 1.54 (0.81-2.93), and age ≥ 80 years, H.R. 3.53 (1.55-8.04). Similarly, the risk of cardiovascular mortality with elevated liver enzymes was also modified by, and increased with age (test for interaction p=0.02); age 70-79, H.R. 3.15 (1.37-7.23), age ≥ 80, H.R. 6.86 (2.44-19.30).

Conclusion: In community dwelling elderly persons, an elevation in both ALT and GGT are associated with an excess risk of all-cause and cardiovascular mortality which increases with age.

Keywords: liver, population, cohort, survival, biomarkers, ageing

Introduction

Elevations in alanine transaminase (ALT) and gamma glutamyltransferase (GGT) are now recognised as robust markers of impaired metabolic health. ALT is an enzyme found predominantly in hepatocytes and is normally present in serum at low levels. However, during hepatocyte injury, levels of ALT increase substantially and are a sensitive and reliable marker of hepatic inflammation(1). GGT is less specific than ALT for liver disease, however sustained elevations in GGT in association with ALT indicate hepatic inflammation; the commonest cause is nonalcoholic fatty liver disease (NAFLD)(2). Cross sectional studies have previously indicated an association between elevated liver enzymes above standard laboratory reference ranges, and cardiometabolic disorders including diabetes(3-5), and these associations were further explored in longitudinal studies, where meta-analyses suggest that an elevation in either ALT or GGT reliably predict incident diabetes(6, 7), metabolic syndrome(8) and all-cause mortality(9, 10). Current literature however, has only studied mortality associations for elevated liver enzymes in isolation, and the association between elevations in both ALT and GGT with mortality outcomes is unknown.

In addition, age may influence the association with mortality. Current evidence suggests that the mortality risk associated with elevated GGT is highest in middle age(11-14), however these studies have enrolled predominantly young to middle aged persons (aged 25-64 years) and there is scant data on the association in older people. Two single studies in exclusively elderly populations with mean age of 70 years have shown a positive linear relationship for GGT and all-cause and cardiovascular mortality(15), however the association of ALT and all-cause mortality remains unclear (16).

In this prospective cohort study, we aimed to examine the association between elevated ALT and GGT and all-cause and cardiovascular mortality in middle aged and older, community dwelling individuals.

METHODS

Study design and population

The Blue Mountains Eye Study (BMES) is a prospective, population based cohort study of vision and other health outcomes in people aged 50 years of age and older in an urban, white Caucasian population west of Sydney, Australia. The survey was conducted among residents of two adjacent postcodes, chosen because they are geographically well defined and have a stable, homogeneous population. The methods have been described in detail elsewhere(17). Briefly, in 1992, door knocking of each household in the area was undertaken to identify and invite all permanent residents born before January 1, 1943; at completion, this census differed from the Australian Census performed 3 months earlier by only 6 people. At study commencement, 4433 eligible residents were invited to participate and 3654 accepted (82.4% participation rate) and underwent clinical interview but blood tests were not collected at this time. In 1997, the study included 2335 individuals who agreed to re-interview and formed the baseline cohort of our study. These individuals underwent a structured questionnaire administered by trained interviewers who recorded demographic characteristics, height, weight and blood pressure. Fasting blood samples were collected for 2061 consenting people. Study follow-up ended in December 2007 when record linkage was performed. The study was approved by the University of Sydney and Sydney West Area Health Service Human Research Ethics Committee and written informed consent was obtained from all participants.

Blood collection

Fasting blood specimens were collected, centrifuged on site and sent within 4 hours to a tertiary level hospital for analysis using commercial kits on an automated analyzer (OCD Fusion 5.1; Ortho Clinical). The coefficient of variation (CV) for repeated measurements in these samples was < 4% for ALT and < 2·8% for GGT. Serum lipids including triglycerides were measured on a Reflotron reflectance photometric analyzer (Boehringer Mannheim Diagnostics; currently, Roche Diagnostics), with CV for triglycerides of 1·4%.

Definition of elevated liver enzymes

We used a sex-specific threshold for elevated ALT and GGT in accordance with laboratory reference ranges and consistent with the threshold used in similar studies (for males, GGT ≥ 51 and ALT ≥ 40, and for females GGT ≥ 33 and ALT ≥ 30)(18). Although a lower threshold for a statistically normal level of ALT has been proposed in cross sectional studies(19), there are currently no data on which threshold predicts long term morbidity and mortality, therefore a more conservative threshold was used.

Ascertainment of outcome (mortality status)

The primary outcomes were all-cause and cause specific (cardiovascular) mortality. To identify those who had died during follow up, record linkage with Australian National Death Index data until December 2007 was undertaken. Causes of death were collected from death certificates completed by the doctor in attendance, coroner or medical examiner, and total and cause-specific mortality were recorded using the *International Classification of Diseases, Ninth Revision* (ICD-9, codes for cardiovascular mortality: (410.0-9, 411.0-8, 412, and 414.0-9). The Australian National Death Index data has high sensitivity and

specificity for all-cause mortality (93.7% and 100% respectively) and cardiovascular mortality (92.5% and 89.6% respectively)(20).

Assessment of other variables

Blood pressure was measured with a calibrated mercury sphygmomanometer. Participants were recorded as having hypertension if blood pressure readings exceeded systolic blood pressure of ≥140 mmHg and diastolic blood pressure of ≥80mmHg, or if they reported the use of anti-hypertensive medication. Participants were considered to have diabetes if they had self-reported doctor diagnosis of diabetes, received treatment in the past or currently, or had a fasting serum glucose > 7.0 mmol/L. Self-reported history of previous myocardial infarctions, previous or current regular smoking, and current alcohol intake (graded as none, 1-2 standard drinks/day, or 3 or more) were recorded. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

Statistical analysis

Baseline characteristics were summarized using frequency tables for categorical variables and quartiles, medians, means and percentiles for continuous variables. Cohorts with elevated or normal–range liver enzymes were compared using X^2 and t-test statistics for categorical and continuous variables respectively. Distribution of exposure, outcome and explanatory variables was assessed graphically, and log transformation of biochemical variables was performed for variables where the distribution was positively skewed. For survival analyses, baseline measurements were considered as the start of biochemical assessment (1997) and follow up continued to date of death or upon censoring (December 31, 2007).

Univariate and multivariate models

For univariate analyses, we used log rank tests to assess the association of survival with the following categorical explanatory variables: sex, smoking status, diabetes, hypertension, previous myocardial infarcts and alcohol intake, and the following continuous explanatory variables: age, body mass index (kg/m^2), total cholesterol (mmol/L) and triglycerides (mmol/L). Univariate Cox proportional hazards models were constructed to estimate the size and direction of hazard ratios for individual variables, and variables with $p < 0.25$ in unadjusted analyses, or variables that were considered potential confounders of the relationship with mortality or cardiovascular mortality such as cardiovascular risk factors (including diabetes, hypertension, BMI, total cholesterol and triglycerides), were included in baseline multivariate models.

Multivariate Cox proportional hazards models were refined using manual backwards elimination until only variables with $p<0.05$ remained. All models were adjusted for alcohol given its known effect on liver enzymes. Effect modification between elevated liver enzymes and (i) age and (ii) sex was assessed using two-way interaction terms and tested in the full model. Linearity of variables was checked by tests for trend and by categorizing into quartiles and plotting the estimated model coefficients against the midpoint of each quartile. Where linearity was uncertain, further assessment using transformation of variables was performed. Linearity of age was assessed by tests for trend, graphical assessment and transformation, and was determined to be linear. To determine if the proportional hazards assumption was met, we tested log (time) dependent covariates sequentially in the model for statistical significance, followed by plotting of Schoenfeld residuals for each variable(21), and visual assessment was undertaken to determine any substantial deviation from the centre. No variables showed evidence of

departure from the proportional hazards assumption. Statistical analyses were performed using SAS 9.3 (SAS institute, Cary, NC).

RESULTS

Baseline cohort

At the first assessment in 1997, there were 2,553 people with data on age, gender and relevant co-morbidities and of these, 2061 (81%) had blood tests available and were used as the baseline cohort (Figure 1). Median follow up was 9.9 years (range 0.2 – 10.9 years), resulting in 20,145 person-years of follow up. Cumulative mortality was 34% (701 deaths), of which 203 (29% of total deaths) were due to cardiovascular disease.

Characteristics of participants

Participant characteristics are shown in Table 1. The prevalence of elevated ALT and GGT above the thresholds of GGT ≥ 51 and ALT ≥ 40 for males, and GGT ≥ 33 and ALT ≥ 30 for females was 5.2% (n=108). The cohort with elevated liver enzymes were more likely to be diabetic (19% vs. 7%, p<0.001), to have a higher mean BMI (29.8 kg/m2 vs. 26.7kg/m^2, p<0.001) and have higher fasting triglyceride levels (1.64 mmol/L vs. 1.32 mmol/L, p<0.001). The proportion of participants who were smokers, hypertensive and had a history of myocardial infarction were similar in both groups.

Elevated ALT/GGT and all-cause mortality

In model building, we tested for significant interactions of elevated liver enzymes with age or sex, and found that the excess risk of mortality with elevated enzymes was not constant, but varied according to baseline age. The hazard ratio for the interaction was positive (H.R. 1.06 95% C.I. 1.01-1.12, p=0.01), indicating that the hazard increased by a multiple of 1.06 for each additional year of age (Table 2), with the plotted hazard curve indicating increasing risk with age (Figure 2). To explore the effect of age, stratified models using age in decades of ≤ 59 years, 60-69, 70-79 and ≥ 80 years (Table 3) were developed, adjusted for significant covariates of sex, smoking status and alcohol. Age-specific estimates for the excess risk and 95% confidence intervals were derived, although confidence intervals are imprecise given they are based on only a subset of the data. Consistent with the results of the main model, the estimated hazard ratio for those with elevated liver enzymes increased with age (H.R. 1.05 (0.53-2.07) for those aged 60-69 years, H.R. 1.54 (0.81-2.93) for 70-79 year olds and H.R. 3.53 (1.55-8.04) for those aged ≥ 80 years (Table 3). There was no evidence of an increase in mortality with elevated liver enzymes among the youngest age group (H.R. 0.46 95% CI 0.06-3.49, for those aged ≤ 59 years). The stratum specific estimates, when plotted against the median age for that stratum, agree closely with the estimated hazard ratios derived from the main model as displayed in Figure 2.

Elevated ALT/GGT and cardiovascular mortality

During model building for cardiovascular mortality, there was also evidence of age modifying the risk of elevated liver enzymes (test for interaction H.R. 1.10, 95% C.I. 1.02-1.20, p=0.02), indicating that the hazard increased by a multiple of 1.10 for each additional year of age. To explore the effect of age on cardiovascular mortality, stratified models using age in decades (≤ 59 years, 60-69, 70-79 and ≥ 80 years) were developed and adjusted for significant covariates of sex and alcohol (Table 3). Age-stratified models did not show increased risk in the youngest age group (for age 60-69 years, H.R. 0.50 (0.07-3.70),

however increased risk was seen in those aged 70-79 years, with a H.R. 3.15 (1.37-7.23) and for those ≥80 years or older, H.R. 6.86 (2.44-19.30) (Table 3). The hazard curve is shown in Figure 2, and if stratum specific estimates are plotted against the median age in that stratum, the estimates are closely approximated and indicate that the excess risk for cardiovascular mortality begins approximately at age 66 years. The slope of the hazard curve for cardiovascular mortality is steeper than that for all-cause mortality, reflecting the larger interaction estimate.

DISCUSSION

These data, from a large prospective cohort study of community dwelling individuals, suggest that middle aged and older persons with elevations in both ALT and GGT have an excess risk of all-cause and cardiovascular mortality that is age-dependent. To explore the effect of age, we stratified the cohort by age per decade and found an increasing risk of all-cause mortality starting at age 60-69 and increasing steadily by decade. While the hazard ratios increased per decade, a statistically significant difference was seen only in the 80 years and older group, reflecting the loss of statistical power that occurs when stratifying into small groups. The hazard curve, plotted from estimated risk per individual year using the model equation, similarly demonstrates increasing risk with age. Regarding cardiovascular mortality risk, a significant increase was seen for those aged 70 years and older and also increased with age. As alcohol is a potential confounder of the relationship between elevated liver enzymes and mortality, it was adjusted for in all analyses but the risk remained independent.

While there are no previous studies of mortality risk in people with elevation in both liver enzymes with which we can compare, these data concur with previous findings of increased mortality with elevation of either GGT or ALT in isolation. Using the same GGT cutoff in a study of 2634 elderly people, Loomba et al found an increase in mortality, with an overall hazard ratio of 1.55 and 1.51 for all-cause and cardiovascular mortality respectively(15), similar to our finding of a hazard ratio for persons aged 70-79 (H.R. 1.54). As this study stratified by age, we found a lower risk for younger persons (HR 1.05 for those aged 60-69 years) and higher for older persons (H.R. 3.53 for those aged 80 and older). A second study in an elderly population of 5,186 people found a similar association of GGT alone and mortality that was linear, and no interaction with age was seen(16). In meta analyses of GGT and all-cause mortality of persons of all ages, there is a relative risk for those in the top third of GGT levels of 1.60 (95% CI 1.42-1.80)(10). Meta-analyses of GGT and cardiovascular mortality show a relationship of similar magnitude (9, 22).

While the relationship between GGT and mortality seems linear, the relationship between elevated ALT alone and mortality appears more complex. A population based study in people aged > 55 years found a J-shaped relationship, where people with ALT levels < 12 or > 35 IU/L had the highest mortality(16); these findings were replicated in a another, smaller study of people aged over 70 where mortality was highest with serum ALT < 12 IU/L(23). Data from a meta-analysis of ALT and all-cause mortality also found a J-shaped association, with higher mortality at serum ALT < 10 IU/L or > 17 IU/L, but no excess risk in between(10). For ALT and cardiovascular mortality, data is conflicting and difficult to interpret, with an inverse relationship seen in some studies(22, 24), while others suggest a positive linear relationship(25). As we examined only persons with elevated ALT in this study, we cannot comment on mortality risk for those with very low ALT. However, the bulk of currently available data suggests

increased mortality risk with elevation of either enzyme alone, and our study extends these findings by demonstrating that increased risk also occurs with elevation of both enzymes, particularly in the very elderly.

The underlying mechanisms for why people with elevated liver enzymes may experience increased mortality are suggested from human and experimental data. GGT metabolizes glutathione, an intracellular antioxidant(26). GGT is upregulated in states of oxidative stress(27) and may influence oxidative events within atherosclerotic plaques causing plaque instability and rupture(28). Elevations in serum GGT levels predict an increased risk of incident metabolic syndrome and diabetes(6), although whether GGT has a causal role is unable to be determined on the basis of observational data. ALT is an enzyme specific for liver inflammation but abundant evidence also supports a predictive role for ALT with metabolic syndrome(8) and diabetes(6, 29). Moreover, it has been assumed that elevated ALT is a consequence of insulin resistance but emerging data proposes that ALT may precede the development of metabolic disorders(30), and further work to disentangle this relationship is awaited.

From a clinical perspective, these data suggest that middle aged and older individuals from the general community who have elevated GGT and ALT have an increased risk of cardiovascular mortality. The presence of elevated liver enzymes should prompt clinical assessment and treatment of modifiable cardiovascular risk factors using standard, evidence based guidelines. From a research perspective, these data raise the possibility that ALT and/or GGT may add incremental value to risk prediction scores. A recent study that has assessed prognostic value of GGT when added to the Framingham risk prediction model found a small but significant improvement in predictive accuracy for cardiovascular disease(31), however other studies have found no incremental benefit with addition of GGT or ALT(14, 32). Given

that cardiovascular death remains the single largest cause of death globally, further work in this area would be informative.

Our choice of threshold for elevated liver enzymes deserves mention. As there is no accepted level of what constitutes normal or abnormal liver biochemistry, we chose gender specific cutoffs of ALT of 30 IU/L for women and 40 IU/L for men based on other large epidemiological studies to allow comparison(33), consistent with lab reference ranges, and supported by the single study that has longitudinally assessed ALT and all-cause mortality risk. That study found that a cutoff of 30 IU/L displayed the best predictive value according to receiver operating curve characteristics(34).

This study has a number of strengths, including near complete capture of a homogeneous population that differed from the national census by only 6 people, an 82.4% initial response rate, high cumulative mortality that increases the study's power, and long duration follow up at a median of 10 years. The dataset had few missing baseline data and included detailed information on confounders. All blood samples were performed fasting and were analysed within 4 hours of extraction, minimizing lab-related measurement error. Furthermore, measurement error in the outcome variable of cardiovascular mortality is likely to be small, given that prior research suggests a high degree of accuracy for correct classification of cardiovascular mortality in this dataset(20). There are a number of potential limitations to our study. Causality cannot be determined on the basis of observational data, however high quality, long duration prospective cohort studies are the best study type to address associations with mortality. Selection bias may also have influenced our results since not all participants agreed to interview and blood tests, however the loss to follow up which may also contribute to selection bias was low. The prevalence of viral hepatitis as a cause for elevated ALT in this Caucasian population is very low (less

than 1%), and even when excluded from demographically similar NHANES-III cohorts, people with viral hepatitis have only accounted for 0.4% of the population(35). Finally, although non-alcoholic fatty liver disease is a common cause of elevated ALT and GGT, we were not able to separate those with NAFLD (diagnosed by liver biopsy or imaging technique) and elevated liver enzymes, and examine mortality outcomes in this subgroup, and this is an important question for future research.

Conclusion

In conclusion, these data from 20,145 person years of follow up, indicate that middle aged and older persons with elevated GGT and ALT have an excess risk of all-cause and cardiovascular mortality that increases with age. These individuals should be targeted for management of metabolic risk factors. Further studies in persons with elevations of both ALT and GGT are desirable to compare to our findings. In addition, research to establish a threshold for liver enzymes above which a significantly increased risk is conferred, would be clinically useful.

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FIGURE LEGENDS

Figure 1. Study cohort

Figure 2. Hazard curve of excess risk of all cause and cardiovascular mortality in those with elevated enzymes compared to those with normal enzymes, according to age.

Table

Table 1: Participant characteristics at baseline for overall cohort and in those with and without elevated enzymes.

	Overall cohort at baseline (n=2061)	People without elevated ALT/GGT (n=1953)	People with elevated ALT/GGT (n=108)	P value
Sex (females)	58% vs.42%	57% vs. 43%	67% vs. 32%	0.04
Age (median, IQR)*	69 (63-75)	70 (63-76)	65 (60-71)	<0.001
Comorbidities (N,%)				
Smoking	1016 (49)	957 (49)	63 (58)	0.09
Hypertension	757 (37)	723 (37)	40 (37)	0.96
Diabetes	171 (8)	137 (7)	21 (19)	<0.001
Previous myocardial infarct	183 (9)	176 (9)	8 (7)	0.38
Anthropometry*				
BMI (kg/m^2)	26.8 (24.2-29.7)	26.7 (24.2-29.6)	29.8(26.7-32.4)	<0.001

Alcohol consumption# (N, %)				
0	553 (24)	24%	18%	0.02^
1-2	1427 (62)	62%	59%	
≥ 3	334 (14)	14%	23%	
Biochemistry*				
ALT (IU/ml)	19 (15-26)	19 (14-24)	45 (38-60)	<0.001
GGT (IU/ml)	22 (16-32)	21 (16-29)	69 (51-118)	<0.001
Fasting triglycerides (mmol/L)	1.34 (0.98-1.83)	1.32 (0.97-1.78)	1.64 (1.2-2.4)	<0.001
Fasting total cholesterol (mmol/L)	5.9 (5.2-6.6)	5.9 (5.2-6.6)	6.05 (5.5-6.8)	0.07

**median with 25th-75th centiles;# standard drinks of alcohol/day where 1 standard drink=10g alcohol.^ overall P value.*

Table 2. Adjusted hazard ratios for all-cause and cardiovascular mortality for those with elevated GGT/ALT compared to those without elevated GGT/ALT.

	All-cause mortality	Cardiovascular mortality
	Adjusted HR (95% CI)	Adjusted HR (95% CI)
Elevated GGT/ALT vs non-elevated#	1.26 (0.82-1.96)	1.29 (0.49-3.44)
Age (per year)*	1.05 (1.01- 1.11)	1.04 (0.95-1.13)
Interaction (Elevated GGT/ALT ×age)	1.06 (1.01 -1.12)	1.10 (1.02-1.20)
Male sex	1.59 (1.35-1.87)	2.42(1.82-3.24)

Smoking (previous or current)	1.34 (1.14 -1.57)	NA
Alcohol (1-2 standard drinks) vs none	0.69 (0.58-0.82)	0.74 (0.55-1.01)
Alcohol (≥3 standard drinks) vs none	0.79 (0.61-1.03)	0.59(0.35-0.99)

#Age centred at the median age of 69 years, therefore this represents the hazard ratio for a 69 year old with elevated GGT/ALT.

**The interaction term represents the multiplicative factor by which the hazard ratio increased for those with elevated GGT/ALT per additional year of age*

Table 3. Adjusted* hazard ratios for all-cause and cardiovascular mortality for people with elevated GGT/ALT compared to those without elevated GGT/ALT, stratified by age in decades.

Age (decade)	Median Age (years)	All-cause mortality	Cardiovascular mortality
		Adjusted Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)
≤ 59	57	0.46 (0.06-3.49)	N/A
60-69	64	1.05 (0.53-2.07)	0.50 (0.07-3.70)
70-79	74	1.54 (0.81-2.93)	3.15 (1.37-7.23)
≥ 80	83	3.53 (1.55-8.04)	6.86 (2.44-19.30)

**Hazard ratios for all-cause mortality adjusted for age, sex, smoking and alcohol intake. Hazard ratios for cardiovascular mortality adjusted for age, sex and alcohol intake*.

Figure 1

Click here to download Figure Figure 1_ study cohort for JGM tiff.tiff

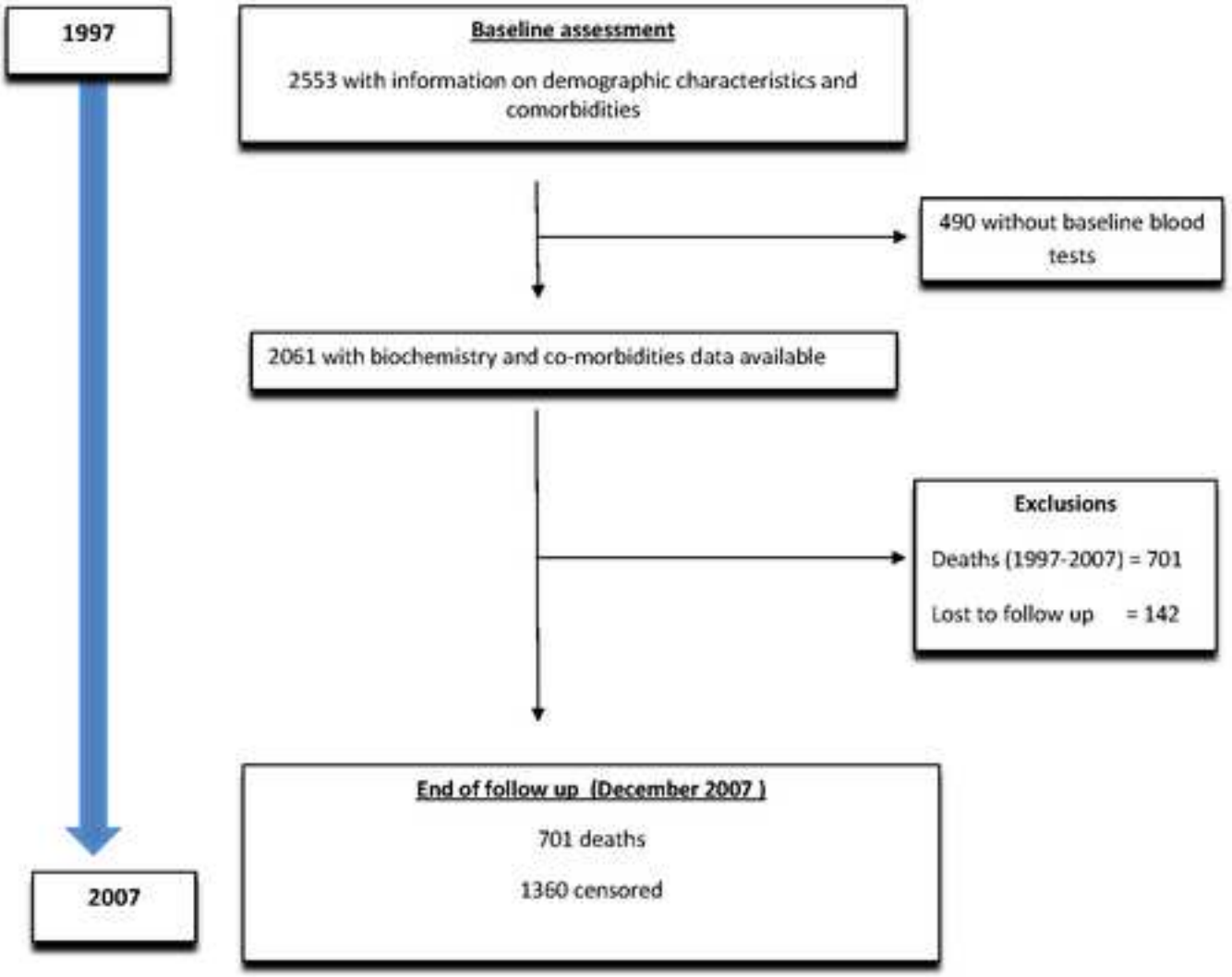
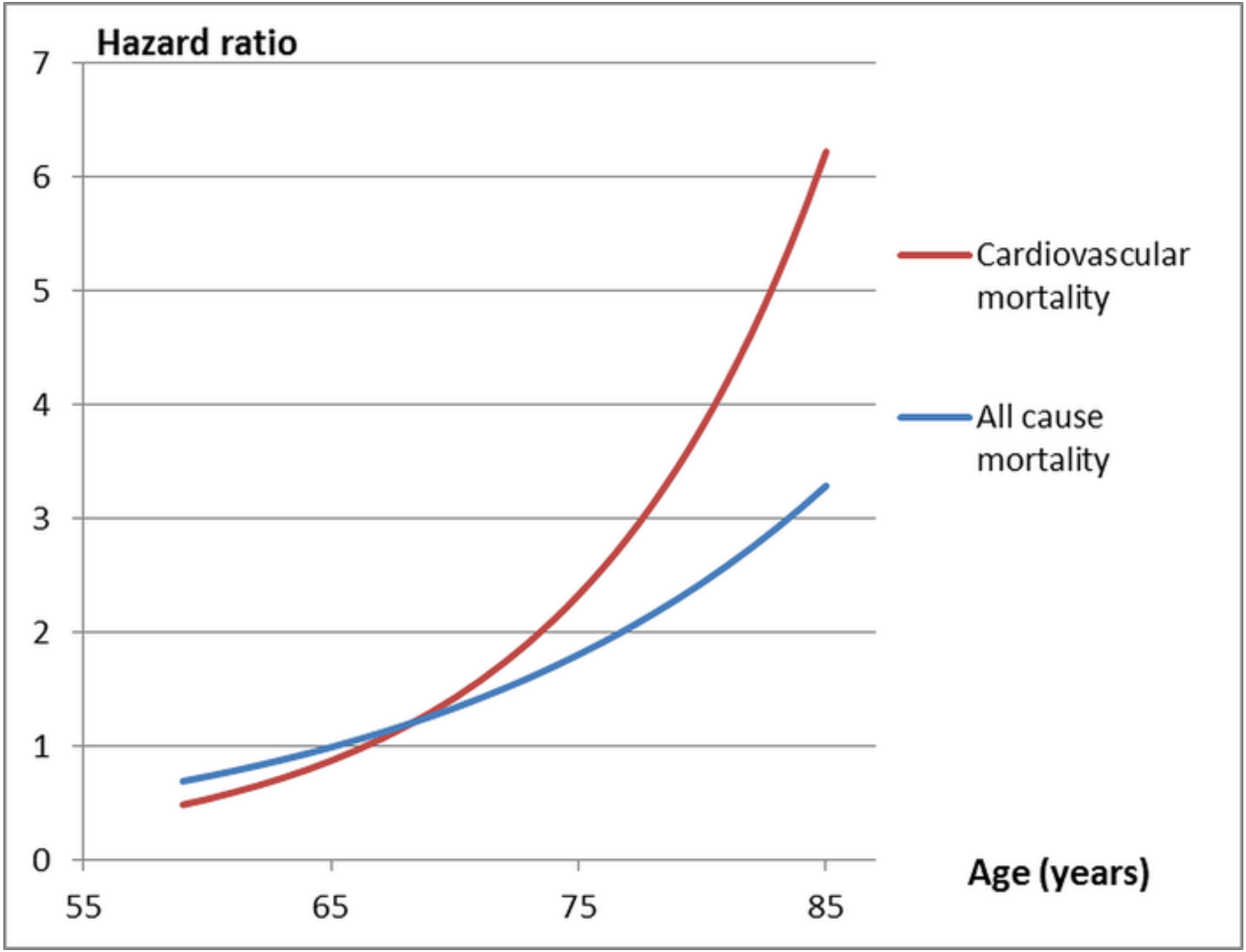


Figure 2

Click here to download Figure Figure 2 ALT GGT hazard curve.tif



THE UNIVERSITY OF SYDNEY

Dr SE Mahady
School of Public Health
University of Sydney
Camperdown NSW 2006 Australia

10th February 2016

Dr Martin H. Floch, MD
Editor, Journal of Clinical Gastroenterology

Dear Dr Floch,

Thank you for considering our study "Elevated liver enzymes and mortality in older individuals: a prospective cohort study" for publication in Journal of Clinical Gastroenterology.

Liver enzymes are a widely and frequently used diagnostic test, and there has been increasing interest in whether they may be useful as biomarkers of incident metabolic diseases and mortality. Elevations in GGT and ALT are associated with increased all-cause mortality, with meta-analyses showing a positive, linear association particularly for GGT. However whether combinations of liver enzymes, such as elevations in both ALT and GGT, offer better predictive accuracy has not been studied.

We utilized data from a large, prospective, population based study of older people (mean age 70 years) to assess the association between elevated enzymes and all-cause and cardiovascular mortality. We performed fully adjusted survival analyses using

baseline liver enzyme measurements, with linkage to national death registries and long duration (median 10 years) follow up.

Our findings suggest that those with elevated enzymes at baseline have an increased risk of mortality at follow up, and the excess risk of mortality increases with age. The nature of the relationship appears to be J-Shaped, with excess, independent risk commencing in the decade 60-69 years. We also found the excess risk for cardiovascular mortality was higher than that for all-cause mortality. This information is useful at a clinical and population level, and provides novel data given that all existing studies have only looked at either enzyme in isolation, and in younger, lower risk populations.

This study was approved by the University of Sydney and Sydney West Area Health Service Human Research Ethics Committee and written informed consent was obtained from all participants. We confirm that this manuscript has not been submitted elsewhere.

Thank you for the opportunity to submit to Journal of Clinical Gastroenterology,

Dr S Mahady on behalf of the co-author team.