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Inverse association of alanine aminotransferase within normal range with prognosis in patients with coronary artery disease

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ABSTRACT

Background: Data regarding the association between alanine aminotransferase (ALT) and prognosis of patients with coronary artery disease (CAD) are limited. The aim of this study was to assess the association of ALT with the prognosis of patients with CAD.

Methods: The study included 9523 patients with angiography-proven CAD who underwent percutaneous coronary intervention. Baseline ALT activity measurements were available for analysis in all patients. The primary outcome was 3-year cardiac mortality.

Results: Patients were divided into three groups: a group with ALT within the 1st tertile (ALT 2.0 U/L to ≤17.0 U/L; n = 3276 patients), a group with ALT within the 2nd tertile (ALT > 17.0 U/L to ≤26.0 U/L; n = 3075 patients) and a group with ALT within 3rd tertile (> 26 U/L to ≤50.0 U/L; n = 3172 patients). Cardiac death (primary outcome) occurred in 441 patients: 201 (7.1%), 126 (4.7%) and 114 (4.0%) of these occurring in patients in the 1st, 2nd and 3rd ALT tertiles, respectively (with percentages representing Kaplan–Meier estimates of 3-year cardiac mortality); adjusted hazard ratio = 1.43, 95% confidence interval 1.11 to 1.85, P = 0.006 calculated for 1 unit decrement in the logarithmic scale of ALT. The multivariable model for cardiac mortality with baseline variables without ALT had a C-statistic of 0.827 [0.801–0.853], P < 0.001, which increased to 0.832 [0.806–0.857], P < 0.001 after incorporation of ALT (P = 0.020).

Conclusions: In patients with CAD, ALT was inversely and independently associated with the risk of 3-year cardiac mortality. Low ALT may reflect cardiovascular risk that is poorly mediated by traditional cardiovascular risk factors.

1. Introduction

Alanine aminotransferase (ALT; EC 2.6.1.2) catalyzes the reversible transfer of amino group from alanine to α-ketoglutarate, playing a key role in the metabolism of amino acids and gluconeogenesis. ALT activity (thereafter as ALT) was detected in serum as early as 1955 by Karmen et al. [1]. ALT is found abundantly in the cytoplasm of hepatocytes at activity levels 3000 times higher than in serum [2]. ALT is also detected in kidney, heart, brain, skeletal muscle, pancreas, spleen, lung and erythrocytes [3]. Serum ALT is regarded as a reliable and sensitive test of liver disease and an indicator of overall health [2]. Available data suggest an association between ALT and cardiovascular disease (CVD) or mortality but this issue remains incompletely investigated and controversial. Prospective cohort studies that have investigated the association between circulating ALT and all-cause or cardiac mortality have produced conflicting results, with some of them reporting no association [4–6] and others reporting a positive [7–9], negative [10–14] or U-shaped [12,15,16] association. Recent meta-analyses have reported an overall negative association between ALT and all-cause or CVD-related mortality [17,18]. However, caution is warranted when interpreting their results due to the inconsistent character of the association, highly significant heterogeneity across the studies and variations in the direction of association by age [17], diabetes status [19] or geographic location [18]. Evidence on the association between ALT and the risk of mortality in patients with coronary artery disease (CAD) is rather limited [20]. We undertook this study

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with 3 aims: first, to assess the association between ALT and outcome (cardiac death, all-cause death, nonfatal myocardial infarction, stroke and stent thrombosis) in a large series of patients with CAD; second, to assess whether the association between ALT and outcome differs in various subgroups of patients, and; third, to investigate whether ALT improves risk prediction for mortality when added into risk prediction models for mortality alongside CVD risk factors.

2. Methods

2.1. Patients

The study included 9523 patients with coronary angiography-confirmed CAD who underwent percutaneous coronary intervention (PCI) in 2 university hospitals in Munich, Germany. The source sample included 18,334 consecutive patients admitted in these institutions between January 2000 and January 2011 [21]. Patients with acute infections, known malignancies, known hepatobiliary disease, alcohol abuse, patients on dialysis at the time of hospital admission, patients who developed cardiogenic shock (on admission or during the hospital course) and those with an ALT level > 50 U/L were excluded. In total, 9523 patients with an ALT ≤50 U/L (the upper limit of reference range in our laboratory) were included in this study. The criteria for CAD diagnosis included: chest pain suggestive of myocardial ischemia, history of myocardial infarction or coronary intervention, positive exercise testing or stress imaging or biomarker elevation (cardiac troponin or creatine kinase myocardial band) with CAD confirmed by coronary angiography, defined as coronary artery stenoses with ≥50% lumen narrowing in at least one major coronary artery. By design, the study represents a retrospective analysis. The study conforms to the Declaration of Helsinki.

2.2. Procedure, drugs and definitions

Coronary angiography and PCI were performed as per standard practice. Periprocedural pharmacological therapy included pretreatment with antithrombotic drugs, clopidogrel (loading dose 600 mg) and intravenous aspirin (325–500 mg) and anticoagulation with unfractional heparin or bivalirudin. At discharge, dual antithrombotic therapy with aspirin (80–325 mg/day continuously) and a P2Y12 inhibitor (mostly clopidogrel) for at least 6 months in patients with stable CAD or 12 months in patients with acute coronary syndromes was prescribed. Other cardiac medications were prescribed at the discretion of the treating physician. CVD risk factors were defined according to the following criteria: arterial hypertension - active treatment with antihypertensive medications or documentation of a systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg on at least two separate occasions; hypercholesterolemia - a documented total cholesterol of ≥220 mg/dL or prior or ongoing therapy with lipid-lowering drugs; type 2 diabetes - a history of diabetes with active treatment with insulin or oral hypoglycemic agents or documentation of an abnormal fasting blood glucose (≥126 mg/dL) or glucose tolerance test (≥200 mg/dL) according to World Health Organization criteria for diabetes [22] or a blood glucose > 200 mg/dL at any time; current smoking - regular consumption of any type of tabacco in the last 6 months. Body mass index was calculated using patients' weight and height measured during the hospital course. Left ventricular ejection fraction was calculated using the area–length method on left ventricular angiograms. Angiographic analyses were performed in the core angiographic laboratory by staff members blinded to patients' clinical data. The glomerular filtration rate was estimated according to the Modification of Diet in Renal Disease (MDRD) Eq. [23].

2.3. Biochemical measurements

Blood for ALT measurement was taken on admission (before angiography). ALT was measured in lithium-heparin plasma samples by an IFCC-standardized (37 °C) coupled optical enzyme-assay (with pyridoxal-5′-phosphate activation) on the automatized cobas c 501® system (Roche Diagnostics GmbH, Mannheim, Germany). The principle of the ALT assay is based on the ALT catalyzed reaction of L-alanine with 2-oxoglutarate producing pyruvate and L-glutamate. Pyruvate is reduced by lactate dehydrogenase (using NADH as reducing agent) to L-lactate and NAD^+. The rate of NADH oxidation is directly proportional to the rate of formation of pyruvate and thus ALT activity (determined by reduction of extinction at 340/700 nm). The measuring range of this method is 5–700 U/L. Reference ranges in our laboratory are 10–50 U/L for adult men and 10–35 U/L for adult women. Blood glucose, high-sensitivity C-reactive protein, total cholesterol, low-density lipoprotein, high-density lipoprotein and aspartate aminotransferase were measured using commercially available assays. Serum creatinine was measured using a kinetic colorimetric assay based on the compensated Jaffe method. All biochemical measurements were performed by laboratory personnel unaware of the patients' clinical data.

2.4. Study outcomes and follow-up

The primary outcome was 3-year cardiac mortality. Secondary outcomes were: all-cause mortality; nonfatal myocardial infarction; stroke; and definite stent thrombosis. Cardiac deaths were defined according to Academic Research Consortium criteria and included any death due to a proximate cardiac cause, e.g. myocardial infarction, low-output failure, fatal arrhythmia, unwitnessed death, death of unknown cause, and all procedure-related deaths, including those related to concomitant treatment and definite stent thrombosis [24]. Mortality data were obtained from hospital records, death certificates, insurance companies and registration of address office or phone contact with the patient's relatives or referring physician. Nonfatal myocardial infarction was defined using electrocardiographic criteria (new abnormal Q waves) or documentation of creatine kinase myocardial band activity elevation > 2 times (> 3 times within the first 48 h after PCI) the upper limit of normal. Stroke was defined as an acute neurological event of at least 24-h duration, with focal signs and symptoms and without evidence supporting any alternative explanation. The diagnosis of stroke was confirmed by brain imaging tests (computed tomography or magnetic resonance imaging). Definitive stent thrombosis was defined according to Academic Research Consortium criteria [24]. Follow-up included a telephone interview at 1 month, a hospital visit at 6 months and telephone interviews at 1, 2 and 3 years. All follow-up information and adjudication of events was performed by dedicated personnel unaware of ALT levels or clinical data.

2.5. Statistical analysis

Patients are categorized in groups according to ALT tertiles. Data are presented as median with 25th to 75th percentiles or counts and proportions (%). The normality of distribution of continuous data was assessed using the Kolmogorov–Smirnov test. Continuous data are compared using the Kruskal–Wallis rank-sum test. Categorical data are compared using the chi-square test. Correlates of ALT are assessed using the multivariable linear regression model. Survival analysis is performed using the Kaplan–Meier method and univariable Cox proportional hazard-model. The multivariable Cox proportional hazard model is used to test the association between ALT and mortality. Covariates entered into the model were selected using the Least Absolute Shrinkage and Selection Operator (LASSO) regression method (R-package “glmnet”, version 2.0–13). The following variables were entered into the model: ALT, age, sex, diabetes, body mass index, arterial hypertension, history of hypercholesterolemia, previous myocardial infarction, current smoking, CAD extent, clinical presentation (acute coronary syndrome or stable CAD), glomerular filtration rate, C-reactive protein, low-density lipoprotein cholesterol, high-density

lipoprotein cholesterol, fasting glucose, total stented length and left ventricular ejection fraction. Due to the skewed distribution, ALT was entered into the model after logarithmic transformation and the risk estimates related to this variable were calculated per unit of logarithmic scale. Missing baseline data are imputed by predictive mean matching (R-package "mice", version 2.46). Interaction testing was performed to assess whether the association of ALT with cardiac mortality differs across patient subgroups. All variables that showed a significant association with cardiac mortality in the multivariable Cox model plus sex were entered into the interaction testing analysis. The C-statistic of Cox proportional hazard model with baseline variables only and with baseline variables plus ALT was calculated to assess whether incorporation of ALT improves the discriminatory power of the model with respect to prediction of cardiac mortality. The same variables as for the model for cardiac mortality were entered into the model(s) used to calculate the C-statistic. Bootstrapping method (400 samples) was used to calculate the confidence interval of the C statistic(s), enable their comparison and assess the stability of the results. The statistical analysis was performed using the R 3.4.0 Statistical Package (The R foundation for Statistical Computing, Vienna, Austria). A two-sided $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Baseline data

Overall, 9523 patients with CAD were included. The tertile values of ALT were used to divide patients into 3 groups: a group with ALT within the 1st tertile (ALT 2 U/L to ≤17.0 U/L; n = 3276 patients), a group with ALT within the 2nd tertile (ALT > 17.0 U/L to ≤26.0 U/L; n = 3075 patients) and a group with ALT within 3rd tertile (> 26 U/L to ≤50.0 U/L; n = 3172 patients). Baseline data are shown in Table 1.

Table 2
Correlates of alanine aminotransferase activity level obtained from the multiple linear regression model.

Variable[a]	Coefficient[b]	P value
Age	−0.12	< 0.001
Female sex	−2.05	< 0.001
Body mass index	0.29	< 0.001
Current smoker	−1.25	0.020
History of hypercholesterolemia	1.88	< 0.001
Previous myocardial infarction	−2.08	< 0.001
Previous coronary artery bypass surgery	−0.85	0.020
Presentation with an acute coronary syndrome	1.94	< 0.001
Estimated glomerular filtration rate	0.09	< 0.001
Fasting glucose	0.02	< 0.001
Low-density lipoprotein cholesterol	−0.02	< 0.001
Left ventricular ejection fraction	−0.05	< 0.001

[a] Variables entered into the model were: age, female sex, body mass index, diabetes, arterial hypertension, history of hypercholesterolemia, previous myocardial infarction, previous coronary artery bypass surgery, clinical presentation, extent of coronary artery disease, C-reactive protein, glomerular filtration rate, fasting glucose, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and left ventricular ejection fraction. C-reactive protein showed a strong trend toward an association with ALT (coefficient: -0.00377; P = 0.062).

[b] Coefficients denote the change in ALT level per unit change in the independent variable. The negative sign before the coefficients shows the inverse correlation between the independent variable and the ALT level.

All characteristics but diabetes and extent of CAD differed significantly according to the ALT tertiles. Coronary stents were implanted in all patients: bare-metal stents in 3243 patients (34.0%) and drug-eluting stents in 6280 patients (66.0%). At hospital discharge, a beta-blocker, angiotensin converting enzyme inhibitor or angiotensin II receptor blocker and a statin were prescribed in 94%, 93.4% and 92.5% of the

Table 1
Baseline data.

Variable	Alanine transaminase tertiles			P value
	1 (n = 3276)	2 (n = 3075)	3 (n = 3172)	
Age (years)	70.4 [62.3–77.3]	67.9 [60.2–74.6]	64.7 [56.9–72.1]	< 0.001
Female sex	1093 (33.4)	767 (24.9)	586 (18.5)	< 0.001
Body mass index (kg/m^2)[a]	26.0 [23.9–28.6]	26.7 [24.5–29.4]	27.6 [25.0–30.4]	< 0.001
Type 2 diabetes	816 (24.9)	769 (25.0)	837 (26.4)	0.318
Arterial hypertension	2329 (71.1)	2176 (70.8)	2135 (67.3)	0.001
Current smoker	557 (17.0)	560 (18.2)	650 (20.5)	0.001
History of hypercholesterolemia (≥220 mg/dl)	1931 (58.9)	2072(67.4)	2150 (67.8)	< 0.001
Previous myocardial infarction	864 (26.4)	745 (24.2)	689 (21.7)	< 0.001
Previous coronary artery bypass surgery	479 (14.6)	410 (13.3)	385 (12.1)	0.014
Stable coronary artery disease	2093 (63.9)	1730 (56.3)	1645 (51.9)	< 0.001
Acute coronary syndrome	1183 (36.1)	1345 (43.7)	1527 (48.1)	< 0.001
Extent of angiographic coronary artery disease				0.312
1	768 (23.4)	697 (22.7)	775 (24.4)	
2	959 (29.3)	891 (29.0)	946 (29.8)	
3	1549 (47.3))	1487 (48.3)	1451 (45.8)	
Multivessel disease	2508 (76.6)	2378 (77.3)	2397 (75.6)	0.256
C-reactive protein (mg/L)	5.2 [1.3–13.1]	2.6 [1.0–7.4]	2.6 [1.0–7.6]	< 0.001
Serum creatinine (mg/dL)	1.00 [0.90–1.20]	1.00 [0.80–1.10]	0.92 [0.80–1.10]	< 0.001
Estimated glomerular filtration rate (mL/min)	65.5 [53.5–77.2]	73.6 [60.2–86.9]	77.0 [64.0–91.6]	< 0.001
Alanine aminotransferase (U/L)	12.5 [10.0–15.0]	21.5 [19.2–23.7]	33.6 [29.3–39.8]	< 0.001
Aspartate aminotransferase (U/L)	14.3 [9.0–21.0]	23.0 [18.2–28.6]	28.7 [23.0–39.5]	< 0.001
Total cholesterol (mg/dL)	198.0 [165.0–230.0]	190.0 [162.0–224.0]	191.0 [160.0–225.2]	< 0.001
Low-density lipoprotein-cholesterol (mg/dL)	119.0 [92.7–148.0]	111.0 [86.7–142.0]	113.0 [85.7–142.0]	< 0.001
High-density lipoprotein-cholesterol (mg/dL)	48.5 [40.4–58.7]	48.4 [40.1–59.3]	47.0 [38.9–56.3]	< 0.001
Fasting glucose (mg/dL)	100.0 [88.2–122.0]	105.5 [94.6–124.0]	107 [96.7–129.0]	< 0.001
Left ventricular ejection fraction (%)	58.0 [47.0–65.0]	58.0 [48.0–63.0]	57.0 [47.0–62.0]	< 0.001
Total stented length (mm)	20.0 [16.0–28.0]	23.0 [18.0–30.0]	23.0 [18.0–30.0]	< 0.001

Data are presented as median [25th; 75th percentiles] or number of patients (%).

[a] The following variables (number of missing data; percentage) were incomplete: body mass index (n = 32; 0.04%), C-reactive protein (n = 222; 2.3%), serum creatinine (n = 129; 1.3%), total cholesterol (n = 203; 2.1%), low-density lipoprotein cholesterol (n = 405; 4.0%), high-density lipoprotein cholesterol (n = 394; 4.0%) and left ventricular ejection fraction (n = 1124; 11.8%). The remaining data were complete.

patients, respectively.

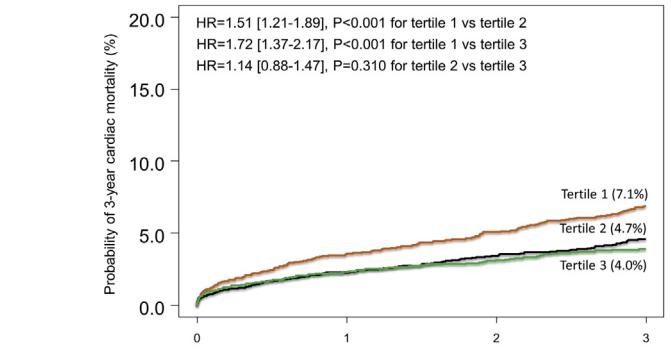
3.2. Correlates of ALT

Correlates of ALT were assessed using the multiple linear regression model. Variables that showed an independent association with ALT and the direction of association are shown in Table 2. Age, female sex, current smoker, previous myocardial infarction, previous coronary artery bypass surgery, low-density lipoprotein cholesterol and left ventricular ejection fraction were inversely associated with ALT level. Conversely, body mass index, history of hypercholesterolemia, presentation with an acute coronary syndrome, glomerular filtration rate and fasting glucose were positively associated with ALT level.

3.3. Clinical outcomes

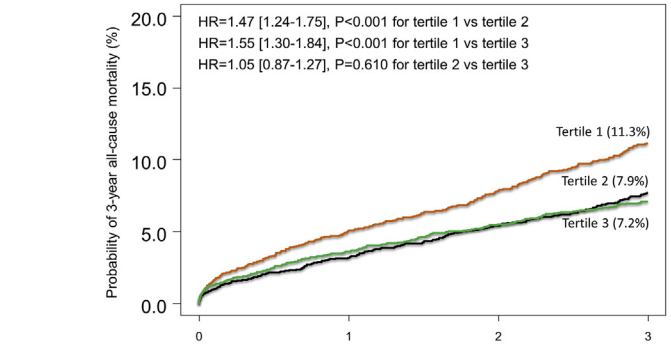
Patients were followed up to 3 years after PCI (median 2.9 [2.2–3.0] years). Cardiac death (primary outcome) occurred in 441 patients: 201 (7.1%), 126 (4.7%) and 114 (4.0%) in patients in the 1st, 2nd and 3rd ALT tertiles, respectively (with percentages representing Kaplan–Meier estimates of 3-year cardiac mortality); unadjusted hazard ratio (HR) = 1.51, 95% confidence interval [CI] 1.21 to 1.89], P < 0.001 for ALT tertile 1 vs. tertile 2; HR = 1.72 [1.37–2.17], P < 0.001 for ALT tertile 1 vs. tertile 3; and HR = 1.14 [0.88–1.47], P = 0.310 for ALT tertile 2 vs. tertile 3 (Fig. 1). All-cause death occurred in 735 patients: 323 (11.3%), 208 (7.9%) and 204 (7.2%) in patients in the 1st, 2nd and 3rd ALT tertiles, respectively; unadjusted HR = 1.47 [1.24–1.75], P < 0.001 for ALT tertile 1 vs. tertile 2; HR = 1.55 [1.30–1.84], P < 0.001 for ALT tertile 1 vs. tertile 3; and HR = 1.05 [0.87–1.27], P = 0.610 for ALT tertile 2 vs. tertile 3 (Fig. 2). The occurrence of nonfatal myocardial infarction, stroke or definite stent thrombosis appears to differ little according to ALT tertiles. Nonfatal myocardial infarction occurred in 356 patients: 126 (4.1%), 117 (4.0%) and 113 (3.8%) in patients with ALT within the 1st, 2nd and 3rd tertiles (P = 0.530); stroke occurred in 140 patients: 47 (1.6%), 53 (1.9%) and 40 (1.4%) in patients with ALT within the 1st, 2nd and 3rd tertiles (P = 0.370) and definitive stent thrombosis occurred in 99 patients: 35 (1.1%), 34 (1.2%) and 30 (1.1%) in patients with ALT within the 1st, 2nd and 3rd tertiles (P = 0.900).

To obtain a more detailed view of the relation between ALT and the risk of mortality, a decile-based analysis was performed (Fig. 3). There appears to be an inverse relationship between ALT level and the risk of cardiac and all-cause mortality, with a progressive increase in the risk of mortality with decreasing ALT deciles (P for trend < 0.001 for cardiac and all-cause mortality). Notably, the risk of noncardiac mortality



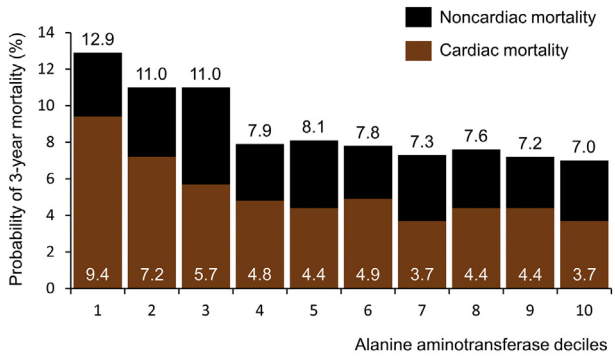
Patients at risk				
ALT tertlie 1	3276	2862	2382	2058
ALT tertile 2	3075	2779	2297	1810
ALT tertile 3	3172	2856	2383	1897

Fig. 1. Kaplan-Meier curves showing 3-year cardiac mortality. ALT = alanine aminotransferase; HR = hazard ratio.



Patients at risk				
ALT tertlie 1	3276	2862	2382	2058
ALT tertile 2	3075	2779	2297	1810
ALT tertile 3	3172	2856	2383	1897

Fig. 2. Kaplan-Meier curves showing 3-year all-cause mortality. ALT = alanine aminotransferase; HR = hazard ratio.



ALT activity (U/L)	2.0 to 10.1	>10.1 to 13.1	>13.1 to 16.1	>16.1 to 18.8	>18.8 to 21.3	>21.3 to 24.1	>24.1 to 27.4	>27.4 to 32.0	>32.0 to 38.3	>38.3 to 50.0
All-cause deaths	116	84	101	59	67	66	58	63	61	60
Cardiac deaths	85	55	51	36	37	42	29	36	37	33
Number of patients	1025	881	1035	881	940	974	940	946	952	949

Fig. 3. Three-year cardiac and all-cause mortality according to deciles of alanine aminotransferase (ALT). Numbers on the top of the bars display Kaplan-Meier estimates of all-cause mortality. Numbers within the brown bars display Kaplan-Meier estimates of cardiac mortality in each ALT decile. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

appears to differ little according to ALT deciles and the pattern of the association of ALT with all-cause mortality is almost entirely driven by the association between ALT and cardiac mortality.

3.4. Results of multivariable analysis

After adjustment in the Cox proportional hazards model (see methods for variables that were entered into the model), low ALT was independently associated with the risk of 3-year cardiac mortality (adjusted HR = 1.43 [1.11–1.85], P = 0.006 calculated for 1 unit decrement in the logarithmic scale of ALT). The association between ALT and all-cause mortality was attenuated (adjusted HR = 1.19 [0.97–1.45], P = 0.095 calculated for each unit decrement in the logarithmic scale of ALT). The full results of the Cox proportional hazards model applied to assess correlates of cardiac and all-cause mortality are shown in Table 3. If ALT was entered into the model as a category dichotomized at the ALT cut-off of 17 U/L (the upper limit of ALT tertile 1), then ALT ≤17 U/L was independently associated with the risk of cardiac mortality compared with ALT > 17 U/L (adjusted HR = 1.33 [1.02–1.72], P = 0.032).

The discrimination of risk prediction for cardiac mortality by ALT level was assessed by calculating the C-statistic of the multivariable Cox

Table 3
Results of multivariable Cox proportional hazards model applied to assess correlates of 3-year mortality.

Characteristics[a]	Hazard ratio [95% confidence interval]	
	Cardiac mortality	All-cause mortality
Alanine aminotransferase (per 1 unit log decrement)	1.43 [1.11–1.85]	1.19 [0.97–1.45]
Age (per 10-year increment)	1.99 [1.69–2.34]	2.20 [1.94–2.50]
Female sex	0.86 [0.63–1.16]	0.81 [0.64–1.02]
Type 2 diabetes mellitus	1.57 [1.16–2.13]	1.67 [1.33–2.10]
Body mass index (per 5 kg/m^2 increment)	0.87 [0.73–1.02]	0.92 [0.81–1.04]
Arterial hypertension	0.83 [0.63–1.10]	0.90 [0.72–1.12]
Current smoker	1.45 [1.00–2.11]	1.53 [1.16–2.02]
History of hypercholesterolemia	0.81 [0.63–1.06]	0.78 [0.64–0.95]
Previous myocardial infarction	1.04 [0.78–1.37]	1.08 [0.87–1.34]
Clinical presentation (ACS vs. stable CAD)	0.94 [0.72–1.23]	1.04 [0.85–1.27]
Multivessel disease (versus. Single vessel disease)	1.86 [1.21–2.87]	1.29 [0.97–1.71]
C-reactive protein (per 5 mg/L increment)	1.02 [1.01–1.04]	1.02 [1.01–1.03]
Glomerular filtration rate (for 30 mL/min decrement)	1.52 [1.23–1.90]	1.29 [1.11–1.51]
LDL-cholesterol (per 30 mg/dL increment)	1.00 [0.91–1.10]	0.96 [0.89–1.03]
HDL-cholesterol (per 10 mg/dL increment)	0.93 [0.85–1.03]	0.98 [0.91–1.05]
Fasting glucose (per 20 mg/dl increment)	1.00 [0.94–1.07]	0.98 [0.94–1.03]
Total stented length (per 10 mm increment)	1.07 [0.97–1.18]	1.04 [0.96–1.12]
Left ventricular ejection fraction (per 10% decrement)	1.58 [1.45–1.73]	1.51 [1.41–1.61]

ACS = acute coronary syndrome; CAD = coronary artery disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
[a] Covariates in the multivariate model were selected using the Least Absolute Shrinkage and Selection Operator (LASSO) regression method.

proportional hazard model without (with baseline variables only) and with incorporation of ALT (baseline variables plus ALT). The C-statistic of the model without ALT was 0.827 [0.801–0.853], P < 0.001. After incorporation of ALT, the C-statistic increased to 0.832 [0.806–0.857], P < 0.001 (delta C-statistic: 0.005 [0.001–0.10], P = 0.020), showing that the inclusion of ALT in the multivariable model improved the risk prediction for 3-year cardiac mortality.

3.5. Interaction testing analysis

Variables that showed an independent association with cardiac mortality in the multivariable Cox proportional hazard model – age, diabetes, current smoking, multivessel disease, C-reactive protein, glomerular filtration rate and left ventricular ejection fraction - plus sex were categorized into subgroups and tested to determine whether there was an interaction with ALT with respect to prediction of cardiac mortality. Variables were categorized as follows: age (> 68 years [median] vs. ≤68 years), sex (women vs. men), diabetes (yes vs. no), current smoking (yes vs. no), multivessel disease (yes vs. no.), C-reactive protein (> 3 mg/L [median] vs. ≤3 mg/L), glomerular filtration rate (> 60 mL/min vs. ≤60 mL/min) and left ventricular ejection fraction (> 50% vs. ≤50%). ALT was dichotomized at 17 U/L (the upper limit of ALT tertile 1). The results of the interaction testing analysis are shown in Fig. 4. There was an ALT-by-left ventricular ejection fraction interaction showing a higher risk of cardiac mortality associated with low ALT level in patients with left ventricular ejection fraction > 50%. None of other variables showed an interaction with ALT in terms of association with cardiac mortality.

4. Discussion

The main findings of this study are as follows: first, in patients with CAD, ALT within the reference range was independently and linear inversely associated with the risk of 3-year cardiac mortality. An ALT ≤17 U/L was associated with a 33% higher adjusted risk for cardiac mortality compared with ALT values > 17 U/L. Although ALT correlated (inversely) with the risk for all-cause mortality, the association was attenuated after adjustment for CVD risk factors and other relevant clinical variables. Second, ALT was not associated with the 3-year risk of coronary events (nonfatal myocardial infarction or stent thrombosis) or stroke. Third, the association between ALT and the risk of cardiac mortality appears to be directionally consistent in patient groups according to age, sex, diabetes, smoking status, CAD extent, renal function, systemic inflammation and left ventricular ejection fraction. Interaction testing showed that the association between low ALT and the risk of cardiac mortality was stronger in patients with preserved left ventricular function. Fourth, ALT improved significantly but modestly the risk prediction for cardiac mortality when added into the multivariable models containing established CVD risk factors and relevant clinical variables.

Evidence is gradually growing that normal-low ALT values are associated with increased risk of mortality in the middle-to-aged population [10–14]. Recent meta-analyses [17,18] and Mendelian randomization studies [25] seem to support an association between ALT within normal range and the risk of mortality. As far as we know, there is only one study that has investigated the association of ALT with mortality in subjects with established chronic CAD. Peltz-Sinvani et al. [20] retrospectively assessed the association between ALT and all-cause mortality in 6575 patients obtained from the Bezafibrate Infarction Prevention (BIP) registry. Patients with low ALT (defined as ALT < 17 U/L, the lower ALT quartile) had an 11% higher adjusted risk for all-cause mortality over a median of 22.8 years of follow-up. Our study corroborates these findings and adds to them in at least 3 ways: first, our study provides information on cause-specific mortality. We found that the association between low ALT and mortality was almost entirely driven by the association between ALT and cardiac mortality. Second, the association between low ALT and the risk of cardiac mortality was directionally consistent across subgroups according to age, sex, diabetes, smoking, CAD extent, renal function, systemic inflammation and left ventricular function. Third, the use of discriminatory tests (C-statistic) in our study showed that ALT improves prediction of cardiac mortality when added into multivariable models alongside CVD risk factors and other clinical variables.

The mechanisms underlying the association between low ALT and increased risk of mortality remain poorly investigated. Associations between low ALT and advanced age [14], frailty [14], loss of independence [26] and sarcopenia [12] have been suggested. All these factors are known to increase the risk of mortality. In addition, the metabolic consequences of low ALT [26], poor nutritional intake [11], vitamin B6 deficiency [10], liver aging with reduced liver size and blood flow [10,11] were also proposed to explain poor outcomes in subjects with low ALT. Our study identified several correlates of low

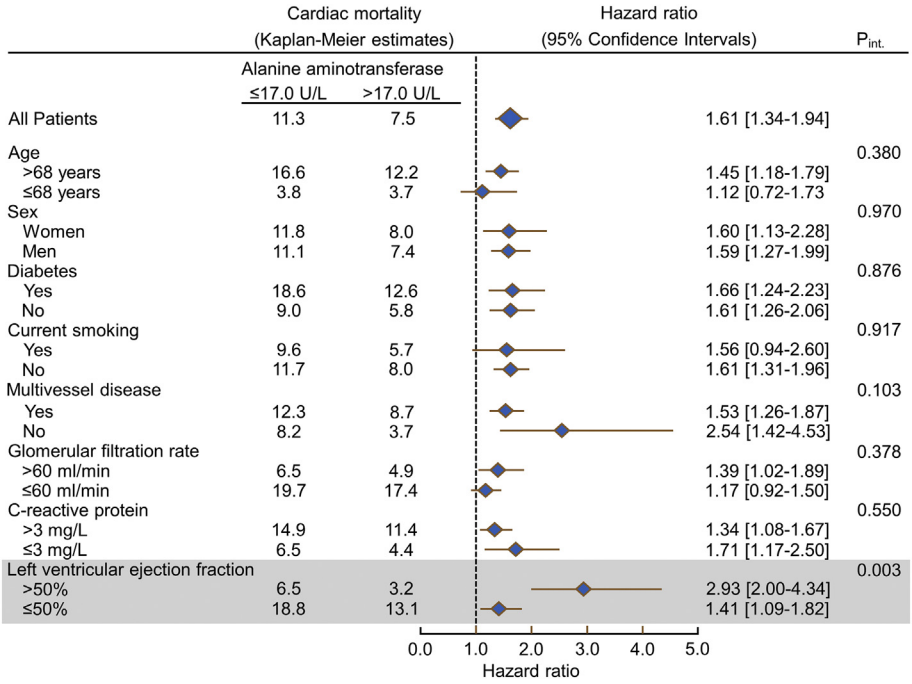


Fig. 4. Cardiac death according to patient subgroups. Deaths are reported as Kaplan-Meier estimates. The gray rectangle marks the ALT-by-left ventricular ejection fraction interaction. P_{int} = P for interaction.

ALT including older age, female sex, lower body mass index, previous myocardial infarction and reduced renal function. Based on our findings and in the context of previous studies, we hypothesize that low ALT may reflect a category of CVD risk that is poorly characterized (if at all) by traditional CVD risk factors. The lack of association (or inverse association) of low ALT with CVD risk factors, the stronger association of ALT with mortality in patients with preserved left ventricular function (potentially mediating CVD risk not related to structural heart disease) and improvement of mortality prediction by ALT would seem to support this hypothesis. The possibility that low ALT may represent CVD risk that is poorly mediated by CVD risk factors may have important implications. Consideration of low ALT as a risk marker may improve CVD risk prediction. This may be important because risk models based on traditional CVD risk factors predict the prospective risk for CAD in approximately 50% of subjects [27] and their prognostic performance decreases with age [28]. The use of low ALT level as risk marker may be particularly helpful in the setting of reverse epidemiology (reverse causality) relationships in which CVD risk factors such as obesity, cholesterol, blood pressure and smoking are inversely related with the patient's outcome in conditions like congestive heart failure or acute coronary syndrome.

The current study has several limitations. First, the study has a retrospective design and was based on a single baseline ALT measurement. Thus, the possibility of residual confounding or ALT variability over time remains unaccounted for. Second, these data are relevant to patients with ALT within the reference range and cannot be extrapolated to assess the association between ALT and mortality in patients with elevated ALT. Third, although our study showed a correlation between low ALT and several factors that may relate to sarcopenia or frailty, the frequency of these conditions remains unknown. Fourth, 3-year follow-up was incomplete in some patients. However, the Kaplan-Meier method used to assess survival may compensate at least partially for incomplete data. Finally, the 3-year follow-up may be short for association studies. Nonetheless, although undesirable, we do not believe that these limitations impact on the main findings of the study.

In conclusion, in patients with confirmed CAD, there is an inverse association between ALT and 3-year cardiac mortality. ALT improved significantly but modestly the risk prediction for cardiac mortality when entered into the models alongside CVD risk factors. A low ALT may reflect a category of CVD risk that is poorly characterized by traditional CVD risk factors. More studies are needed to assess mechanisms of the association between low ALT and increased CVD risk or mortality or improved risk prediction by this biomarker.

Conflict of interests

None.

Funding

None.

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