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Prognostic value of serum gamma-glutamyl transferase activity after myocardial infarction

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Aims Serum gamma-glutamyl transferase activity (γ-GT) is able to catalyse low-density lipoprotein oxidation and has been detected in coronary atherosclerotic plaques. γ-GT has been documented as an independent risk factor for cardiac mortality in middle-aged men. The purpose of this study is to determine the prognostic value of γ-GT in patients with coronary artery disease.

Methods and Results In a prospective study, γ-GT and other cardiac risk factors were evaluated in 469 consecutive subjects with angiographically documented coronary artery disease, using mortality and mortality plus non-fatal myocardial infarction as end-points. γ-GT showed an independent prognostic value beyond known established risk factors in the subgroup of 262 patients with previous myocardial infarction. At a 6-year follow-up, cardiac mortality was 25·2% in patients with γ-GT >40 U . l^{-1} vs 13·9% in those with γ-GT <40 U . l^{-1} (P=0·038). When both cardiac mortality and non-fatal myocardial infarction were considered as end-points, these events were recorded in 32·7% of patients with γ-GT >40 U . l^{-1} and in 20·4% of those with levels <40 U . l^{-1} (P=0·031). Excess mortality and non-fatal infarction in patients with high γ-GT levels were concentrated in the first 2 years of follow-up (P=0·014). The association of γ-GT values >40 U . l^{-1}, previous myocardial infarction, and multiple vessel disease identified a subgroup of 168 patients with the highest risk of cardiac events at 6 years (P=0·024). The relationship between γ-GT levels and cardiac events remained significant after adjustment for cardiac risk factors, and possible confounders, including alcohol consumption. γ-GT did not show significant prognostic value in the 207 patients without previous myocardial infarction.

Conclusion γ-GT is an independent cardiac risk factor in ischaemic patients with established coronary atherosclerosis and previous myocardial infarction.
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Key Words: γ-GT activity, cardiac mortality, myocardial infarction, coronary atherosclerosis.

Introduction

Gamma-glutamyl transferase (γ-GT, E.C. 2.3.2.) is the enzyme responsible for the extracellular catabolism of glutathione, the main thiol antioxidant in mammalian cells[1]. γ-GT is present on the surface of the cell membrane and in serum[1]. It has been suggested recently that γ-GT may have a role in the pathogenesis of atherosclerosis: γ-GT activity has been detected in atheromatous plaques of carotid and coronary arteries[2], and it has been shown that glutathione hydrolysis by γ-GT can trigger iron-catalysed low-density lipoprotein (LDL) oxidation[2], as well as production of reactive oxygen species[3]. These events are known to play a central role in the evolution of atherosclerosis: formation of the fibrous cap[4], apoptosis of cellular elements of the lesion[5], plaque erosion and rupture[6], enhanced platelet aggregation and thrombosis[7].

Serum γ-GT determination is widely used as a diagnostic test for hepatobiliary diseases and alcohol abuse[8]. A few population studies[9–12] have examined the relationship between serum γ-GT and all-cause mortality, focusing on γ-GT as an indicator of alcohol consumption. Serum γ-GT has been found to predict morbidity and mortality independent of alcohol consumption and liver pathology[13]. One study has also addressed the cardiovascular prognostic significance of γ-GT in a large population of middle-aged men, in which top quintile γ-GT values were recognized as an

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independent prognostic marker both for overall and cardiac mortality[10]. In the same study, the observation of a specific influence of serum γ-GT on cardiac mortality in the subgroup of patients with history of previous myocardial infarction suggested a possible relationship with life-threatening complications of underlying coronary artery disease[10].

Another recent study found a prognostic value of serum γ-GT in a subset of patients with ischaemic syndrome, but without ascertained atherosclerotic coronary artery disease[14].

We therefore investigated, over a 6-year follow-up, the prognostic relevance of serum γ-GT activity and its relationship with other cardiovascular risk factors in patients with ischaemic heart disease and angiographically documented coronary artery atherosclerosis.

Methods

We prospectively evaluated 662 consecutive adult patients who underwent diagnostic cardiac catheterization and coronary angiography between 1990 and 1993 at the CNR Institute of Clinical Physiology in Pisa, Italy. Atherosclerotic coronary artery lesions were angiographically diagnosed in 469 patients with documented ischaemic heart disease, who were included in the study population. Thirty-four patients were classified as ischaemic non-atherosclerotic (Prinzmetal's angina without significant atherosclerotic coronary artery disease) and were therefore excluded from the study population. The study complies with the Declaration of Helsinki. The research protocol has been approved by the institutional ethics committee.

At admission, patients were asked to provide information about family history of coronary artery disease, history of angina pectoris, previous myocardial infarction, smoking habit, alcohol consumption, hypertension (defined as a systolic blood pressure of >140 mmHg and a diastolic blood pressure of >90 mmHg in more than one determination, or treatment with antihypertensive drugs), hypercholesterolaemia (defined by a plasma cholesterol level of >220 mg . dl^{-1}), hypertriglyceridaemia (defined by a plasma triglyceride level of >160 mg . dl^{-1}), obesity (body mass index >30), diabetes mellitus (defined by either antidiabetic therapy or a fasting plasma glucose level of >140 mg . dl^{-1} in more than one determination), and non-cardiovascular diseases. Serum cholesterol, triglycerides, glucose, γ-GT and alanine-aminotransferase (ALAT) levels were recorded, body mass index was determined, and arterial pressure was obtained according to WHO guidelines. The normal range of serum γ-GT activity in our laboratory was below 50 U . l^{-1} in males, and 35 U . l^{-1} in females.

All the coronary angiograms were evaluated by cardiologists unaware of the patient risk-factor profile. Coronary artery disease was defined as the presence of significant stenosis, at least 50% of the vessel diameter in any of the main coronary arteries. The extent of coronary artery disease was scored as 0 (absent or minimal atherosclerotic involvement), 1 (single-vessel disease), 2 (two-vessel disease), 3 (three-vessel disease) according to the number of main vessels with significant stenosis. Stenosis of the left main-stem artery without stenosis of the right artery was classified as two-vessel disease. The left ventricular ejection fraction was assessed by ventriculography using the area–length method by modified Simpson's rule.

Follow-up

Follow-up started at hospital admission and continued until study termination (July 1998): information was obtained by independent interviewers, regarding the date of occurrence of myocardial infarction, death or revascularization procedure, directly from patients, relatives, or Institute cardiologists or family doctors, between the time of angiography and July 1998. Information about time and cause of death was obtained from death certificates, post-mortem reports and family doctors. The individual follow-up ended with the first cardiac event, non-cardiac death, or with a revascularization procedure.

Patients who died from non-cardiac causes or following revascularization procedures were considered withdrawn alive. Patients initially treated medically were included in the follow-up until time of revascularization; their subsequent follow-up was censored (withdrawn alive). Six patients were lost to follow-up.

Deaths were classified as due to cardiac disease when caused by acute myocardial infarction, sudden death or congestive heart failure.

Biochemical measurements

Serum γ-GT activity and all other haematochemical data were obtained within the same day from antecubital vein blood samples after overnight fasting, according to the usual clinical laboratory procedures. In particular, γ-GT was assayed at 37 °C using L-γ-glutamyl-3-carboxy-4-nitroanilide as substrate[15] by a Hitachi 717 automatic analyser.

Statistical analysis

Due to the skewness of the serum γ-GT values distribution, a natural logarithmic transformation was applied for statistical analysis when required. Values are presented as mean ± standard deviation (SD). Unpaired t-test was used to compare groups. Multiple regression analysis was applied in order to identify a correlation between serum γ-GT, used as an independent variable, and known risk factors for ischaemic heart disease or

other confounders. Survival curves were analysed using the Kaplan–Meier estimate. The comparison between survival curves was performed using the log-rank and the generalized Wilcoxon tests. Moreover, to identify significant prognostic variables, their individual effect on survival was evaluated with the Cox proportional hazards model[16,17]. According to a stepwise selection process, variables were entered or removed from the regression equation on the basis of a computed significance probability ('maximized partial-likelihood ratio')[17]. This allowed the identification of a subset of variables, all having a significant independent correlation with the incidence of cardiac death or non-fatal infarction. The best cut-off point for the serum γ-GT was 40 U . l^{-1}, obtained by means of parametric Receiver Operating Characteristic (ROC) analysis[18], with cardiac mortality and cardiac mortality plus non-fatal myocardial infarction as the end-points. The 40 U . l^{-1} γ-GT level coincided with the dichotomization cut-point that maximized the hazard ratio obtained from the Cox regression model.

This analysis was performed on the following continuous variables: age, body mass index, serum ALAT, cholesterol, triglycerides, glucose, systolic and diastolic arterial pressure, and left ventricular ejection fraction. Other variables were considered as dichotomic: γ-GT activity (the cut-off value was considered to be 40 U . l^{-1}), sex, family history of ischaemic heart disease, history of previous myocardial infarction, diabetes mellitus, hypercholesterolaemia, arterial hypertension, smoking habit, alcohol consumption, number of diseased vessels (single- vs multiple vessel disease).

The relative risk for each independent variable in the hazard equation was directly proportional to the risk brought by that variable to the model. All relative risks are presented with 95% confidence intervals and all P values are two-sided. P value was considered significant when $<0{\cdot}05$.

Results

Characteristics of the patients

The mean age of the 469 patients with ischaemic heart disease studied (400 males and 69 females) was 59 ± 9 years (range 35–84); 262 (56%) had a diagnosis of previous myocardial infarction, 15 (3%) had a previous revascularization procedure. Diabetes and arterial hypertension were diagnosed in 78 (17%) and 111 (24%) patients, respectively; a total of 354 patients were smokers (75%), 276 were drinkers (59%), with an average daily alcohol intake of 32 ± 19 g. Aspirin was used in 389 patients (83%), calcium-channel blockers in 342 (73%), nitrates in 361 (77%), beta-blockers in 66 (14%), and angiotensin-converting-enzyme inhibitors in 52 (11%). At coronary angiography 187, 166 and 106 patients had one-, two- or three-vessel disease, respectively; 10 patients had left main-stem artery disease. Mean left ventricular ejection fraction was $51{\cdot}0 \pm 13{\cdot}1$%. Twenty-seven percent of patients (126) underwent percutaneous transluminal coronary angioplasty during the follow-up, and 43% (201) coronary artery bypass grafting. The distribution of serum γ-GT in the 469 patients with coronary artery disease was skewed to the right, with a mean value of $39{\cdot}0 \pm 39{\cdot}2$ U . l^{-1}.

Serum γ-GT activity and risk of infarction and cardiac death

Taking into consideration withdrawal at a first cardiac event, non-cardiac death or a revascularization procedure, a median follow-up of 32 months (range 1–105) was achieved in the study population. During the follow-up period, 17 patients had an acute myocardial infarction, and there were 28 cardiac deaths: seven sudden deaths, 10 due to acute myocardial infarction and 11 to congestive heart failure. Thirteen deaths were due to non-cardiac causes and therefore excluded from the analysis. A total of 40 deaths occurred in patients withdrawn alive because of revascularization, 13 were related to cardiac surgery, 14 to cardiac diseases and the remaining 13 to non-cardiac causes.

In terms of the entire 469 patients, at 6 years Kaplan–Meier estimates of mortality or mortality plus non-fatal myocardial infarction were 17·4% and 25·3%, respectively, for patients with a mean γ-GT activity above 40 U . l^{-1} (mean value of the whole population), and 11·6% and 17·9% for those with levels below 40 U . l^{-1} ($P=0{\cdot}091$ and $P=0{\cdot}044$, respectively).

Serum γ-GT activity showed no significant correlation with recognized cardiovascular risk factors; γ-GT correlated only with serum ALAT level ($R=0{\cdot}385$, $P=0{\cdot}01$) and, marginally, with alcohol consumption ($R=0{\cdot}133$, $P=0{\cdot}05$).

Previous myocardial infarction, γ-GT levels and cardiac events

According to the history of previous myocardial infarction, the population was divided into two groups. Serum γ-GT activity showed no significant prognostic value in the group of 207 patients without previous myocardial infarction (age 59 ± 9 years, 39 females).

A significant association between mean serum γ-GT activity and cardiac events was found only in the subgroup of 262 patients with previous myocardial infarction (age 59 ± 9, 30 females), although mean γ-GT values were not significantly different from patients without previous myocardial infarction ($37{\cdot}3 \pm 39{\cdot}0$ vs $41{\cdot}2 \pm 39{\cdot}4$ U . l^{-1}, $P=0{\cdot}29$, respectively).

At 6 years, in patients with previous myocardial infarction and γ-GT 40 U . l^{-1} cardiac mortality was 25·2% vs 13·9% in those with γ-GT <40 U . l^{-1} ($P=0{\cdot}038$). When both cardiac mortality and non-fatal myocardial infarction were considered as end-points,

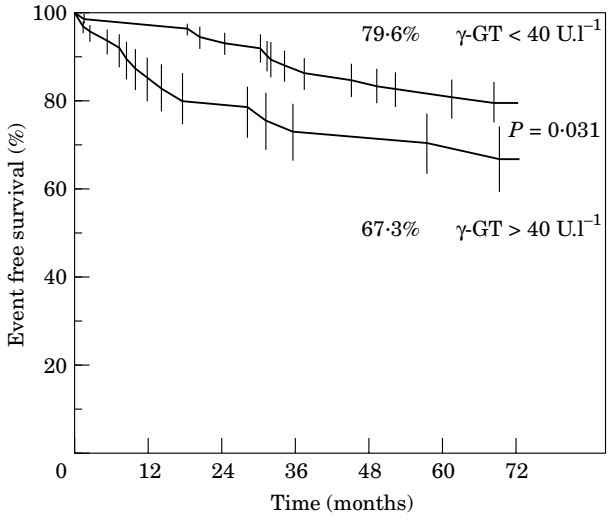

Figure 1 **Event free survival, after 6 years of follow-up, according to serum γ-GT activity among 262 patients with coronary artery disease and history of previous myocardial infarction (185 with γ-GT <40 U . l^{-1} and 77 with γ-GT >40 U . l^{-1}). Vertical lines represent confidence intervals.**

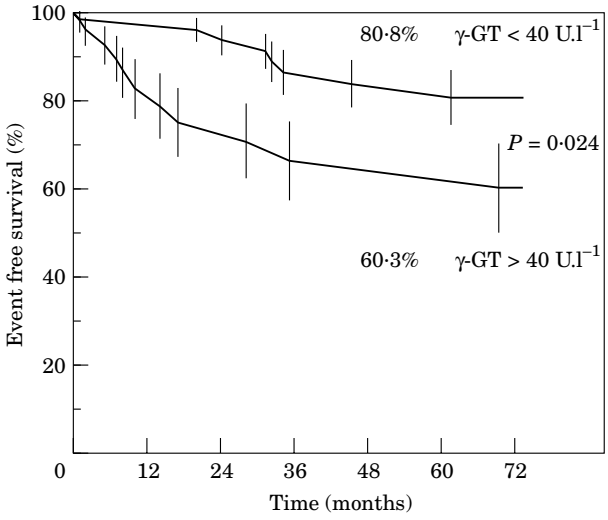

Figure 2 **Event free survival according to serum γ-GT activity in the subgroup of 168 patients with coronary artery disease, history of previous myocardial infarction and multiple vessel disease (119 with γ-GT <40 U . l^{-1} and 49 with γ-GT >40 U . l^{-1}). Vertical lines represent confidence intervals.**

these events were recorded in 32·7% of patients with γ-GT >40 U . l^{-1} and in 20·4% of those with levels <40 U . l^{-1} (P=0·031). Event free patient survival is shown in Fig. 1: excess mortality and non-fatal infarctions in the high γ-GT patients were concentrated in the first 2 years of follow-up (P=0·014), as well as excess mortality alone (P=0·011); from 2–6 years the probability of survival did not differ in the two groups.

According to univariate Cox survival analysis, the strongest predictors of mortality and myocardial infarction were left ventricular ejection fraction (proportional risk 1·03, 95% C.I. 1·01–1·06, P=0·007) and γ-GT (proportional risk 1·97, 95% C.I. 1·01–3·88, P=0·050).

A stepwise regression model selected three variables as independent and additive predictors for cardiac mortality (left ventricular ejection fraction, age, and γ-GT activity), and for both cardiac death and non-fatal myocardial infarction (left ventricular ejection fraction, sex, and γ-GT activity) (Table 1). Alcohol consumption and serum ALAT had no prognostic value in this population. No difference was found when serum cholesterol, glucose, triglycerides, and arterial pressure were considered as continuous variables, instead of as a dichotomized evaluation of the history of hypercholesterolaemia, hypertriglyceridaemia, diabetes, and arterial hypertension.

Furthermore, in 168 patients with previous myocardial infarction, the simultaneous presence of γ-GT values >40 U . l^{-1} and extensive coronary atherosclerosis (i.e. multiple vessel disease) identified a subgroup of patients with the highest risk of cardiac events: at 6 years event-free survival among patients with γ-GT >40 U . l^{-1} was significantly lower as compared to γ-GT <40 U . l^{-1} (P=0·024), as shown in Fig. 2.

When these patients were reevaluated using two incremental serum γ-GT values (25 and 40 U . l^{-1}), the three subgroups showed an increasing risk for cardiac events (<25 U . l^{-1}: 14·3%; 25–40 U . l^{-1}: 27·9%; >40 U . l^{-1}:

Table 1 Independent predictors of mortality or mortality plus non-fatal myocardial infarction for 262 patients with previous myocardial infarction according to a stepwise regression model

	Mortality			Mortality or myocardial infarction		
	Relative risk	95% C.I.	*P* value	Relative risk	95% C.I.	*P* value
γ-GT	2·87	1·18–7·03	0·022	2·17	1·06–4·43	0·036
Sex			ns	3·21	1·34–7·70	0·038
Age	1·15	1·08–1·22	<0·001			ns
LVEF	1·06	1·03–1·10	<0·001	1·03	1·01–1·07	0·007

C.I.=confidence interval; γ-GT=gamma-glutamyl transferase; LVEF=left ventricular ejection fraction.

39·7%, respectively) suggesting a graded response relationship to serum γ-GT activity.

A stepwise regression model applied to this subgroup of patients confirmed γ-GT activity as an independent predictor for cardiac mortality (proportional risk 7·16, 95% C.I. 2·34–21·93, $P=0·001$), or cardiac death plus non-fatal myocardial infarction mortality (proportional risk 3·27, 95% C.I. 1·31–8·11, $P=0·017$), whereas alcohol consumption was found to be protective against mortality (proportional risk 0·19, 95% C.I. 0·06–0·61, $P=0·002$).

Influence of revascularization on the prognostic value of serum γ-GT levels in patients with previous myocardial infarction

To assess the impact of revascularization on the prognostic significance of serum γ-GT activity, the 262 patients with previous myocardial infarction were evaluated for occurrence of cardiac events, without considering revascularization as a cause of withdrawal from follow-up. Cardiac mortality and non-fatal myocardial infarction were recorded in 26·0% of patients with γ-GT >40 U $.1^{-1}$ and in 21·8% of those with levels <40 U $.1^{-1}$ (ns).

Discussion

We found a significant positive association between serum γ-GT activity and the incidence of cardiac death and infarction in ischaemic patients with angiographically documented atherosclerotic coronary artery disease. The prognostic significance of γ-GT was present only in patients with a history of previous myocardial infarction, independent of left ventricular function.

Adjustment for other risk factors for cardiovascular diseases, including age, smoking, hypercholesterolaemia, diabetes, arterial hypertension, obesity, a history of previous myocardial infarction, left ventricular function, extension of coronary artery disease, and for other confounders (including ALAT levels and alcohol consumption) confirmed the independent prognostic value of γ-GT, with a cut-off value of 40 U $.1^{-1}$.

The prognostic significance of γ-GT was particularly evident in a subset of ischaemic patients (36% of the whole population) characterized by the association of multiple vessel coronary artery disease and previous myocardial infarction. In this subgroup, a graded relationship between serum γ-GT level and its predictive value was observed, both for cardiac death and non-fatal infarction, with an increasing risk from 25 to 40 U $.1^{-1}$, within the normality range.

Alcohol consumption exerted a protective influence on survival in this subgroup, confirming previous evidence in patients after myocardial infarction[19] and enhancing the independent prognostic value of γ-GT.

Coronary revascularization abolished the prognostic value of γ-GT, probably counteracting the possible influence of γ-GT on plaque destabilization.

Thus, this study shows that serum γ-GT activity predicts life-threatening events in a subset of patients particularly prone to plaque complications, as indicated by their more extensive coronary atherosclerosis involvement (multiple vessel disease) and history of previous myocardial infarction. In fact, the prognostic significance of elevated serum γ-GT could be strictly related to diffuse and unstable atherosclerotic lesions, more susceptible to complications leading to significant clinical events. This could be confirmed by the temporal relationship between γ-GT levels and cardiac events: excess mortality and non-fatal infarctions in the high γ-GT patients were concentrated within the first 2 years of follow-up, the event curves subsequently becoming parallel.

γ-GT is known as an antioxidant enzyme, in that its activity facilitates the cellular supply of precursors for the resynthesis of the antioxidant GSH[1]. However, recent biochemical evidence shows that γ-GT activity directly participates[2] in the oxidative events related to the evolution of the atheromatous plaque[4–6,20–22]. In vitro observations have shown that γ-GT, in the presence of iron ions, catalyses LDL oxidation[2]: the range of effective γ-GT concentration was 25–100 U $.1^{-1}$[2], the same as that associated with cardiac risk in the present study. Moreover, active γ-GT has been demonstrated in atheromatous plaques, in correspondence with oxidized lipoproteins[2], which might derive from leukocytes or macrophages present within the plaque[23]. However, serum γ-GT is partially adsorbed onto LDL lipoproteins[24], which can carry γ-GT activity inside the plaque (in proportion with serum γ-GT levels), where free iron has also been described[25,26].

The recently described association between increased body iron stores and excess risk of acute myocardial infarction[27] suggests that iron metabolism could influence the predictive value of serum γ-GT.

In conclusion, the serum γ-GT activity level is an independent prognostic index of cardiac death and non-fatal myocardial infarction in patients with coronary atherosclerosis and ischaemic heart disease.

The determination of serum γ-GT, usually considered as a non-specific marker of hepatobiliary disease activity, might have a different implication in patients with ascertained ischaemic heart disease, identifying a subset at highest risk of events, requiring specific and enhanced therapeutic effort.

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