

Opinion Paper

The significance of serum γ -glutamyltransferase in cardiovascular diseases

Alfonso Pompella^{1,*}, Michele Emdin², Claudio Passino² and Aldo Paolicchi¹

¹ Department of Experimental Pathology, University of Pisa Medical School, Pisa, Italy

² Institute of Clinical Physiology, National Research Council (CNR), Pisa, Italy

Abstract

Since early after the introduction of serum γ -glutamyltransferase (GGT) in clinical practice as a reliable and widely employed laboratory test, epidemiological and prospective studies have repeatedly shown that this activity possesses a prognostic value for morbidity and mortality. The association is independent of possibly concomitant conditions of liver disease, and notably, a significant independent correlation of serum GGT exists with the occurrence of cardiovascular diseases (myocardial infarction, stroke). Experimental work has documented that active GGT is present in atherosclerotic plaques of coronary as well as in cerebral arteries. These findings, and the recently recognized functions of GGT in the generation of reactive oxygen species, indicate that serum GGT represents a true marker of cardiovascular diseases and underlying atherosclerosis. Further insights into potential therapeutic interest will probably be derived from studies investigating the origin of GGT activity in plaque tissue.

Keywords: atherosclerosis; glutathione; myocardial infarction; serum γ -glutamyltransferase; stroke.

Introduction

The determination of serum or plasma levels of γ -glutamyltransferase (GGT, EC 2.3.2.2) has represented for decades one of the basic biochemical tools for evaluation of liver function. The activity of GGT in blood can provide a sensitive indication of alterations of hepatic tissue having pathological implications. As such, serum GGT activity has been the parameter of choice, e.g., for the monitoring of alcohol abuse. However, despite its wide acceptance as a routine chemical pathology test, GGT activity has not yet been fully characterized as far as its precise physiological function(s).

*Corresponding author: A. Pompella, MD, PhD, Dipartimento di Patologia Sperimentale BMIE, Scuola Medica, Università di Pisa, Via Roma 55, 56126 Pisa, Italy
Phone: +39-050-2218537, Fax: +39-050-2218557,
E-mail: apompella@biomed.unipi.it

Although GGT in blood is used as a diagnostic marker for liver damage, most research on GGT has been focused at the cellular level. The ability of cellular GGT to effect the catabolism of the antioxidant glutathione – thus playing a pivotal role in the metabolism of low-molecular-weight thiols – has led to the view that GGT activity is related to cellular antioxidant/antitoxic systems. The latest results in the field indicate, however, that the relationship of GGT activity with cellular redox processes is more complex than has been previously believed. As detailed below, recent findings concerning cellular GGT function have provided novel insights into the patho-physiological significance of GGT activity in blood. While the use of GGT as a monitor of liver function holds true and remains important, our perspective can now be widened to include other diseases that are associated with alterations of serum GGT, such as cardiovascular diseases in the first instance. Previous observations will probably find reasonable explanation, and the body of information that has accumulated will likely form the basis for future critical developments.

The functions of GGT activity at the cellular level

GGT activity is critical for the maintenance of adequate intracellular levels of reduced glutathione (GSH), a major antioxidant involved in several important defense mechanisms. Because GSH is poorly transported across cell membranes, its intracellular levels depend on a balance between its consumption and its de novo synthesis, and the latter in turn depends on an adequate supply of precursor amino acids. In several cell types, this is accomplished (for the most part) through GGT activity. GGT is a membrane-bound enzyme, with its active site oriented towards the outer surface of the cell. The enzyme participates in the salvage of extracellular GSH by catalyzing the first step in its degradation, the hydrolytic release of the cysteinyl-glycine and glutamate moieties that can be transported across the membrane to the cytoplasm, where they can enter the GSH synthetic pathway. Thus, GGT has a central role in cellular “GSH cycling” (1).

GGT is constitutively expressed in several organs and tissues (2). In addition, GGT is reexpressed at high levels in malignant or pre-malignant lesions, e.g., liver preneoplastic nodules induced by chemical carcinogens, hepatoma cell lines, and several human spontaneous neoplasms and metastases (reviewed in ref. (3)). Elevation of serum GGT levels is a common

event in liver cancer, both primary and metastatic; moreover, it has been shown (immunologically) that serum GGT is elevated in patients with malignancies of other origin (4). Due to its role in the reconstitution of intracellular GSH, GGT can be regarded as a member of the cellular antioxidant systems; indeed, cells that express high levels of GGT have increased resistance to toxic and prooxidant injury (5–9). The induction of GGT activity has been consistently observed in tissues subject to oxidative stress, notably the lungs (10–12).

A change of perspective: GGT activity as a prooxidant factor

The significance of GGT in redox homeostasis at a cellular level has been highlighted following novel insights from studies showing its ability to play a prooxidant role under selected conditions. The key issue in this alternative function of GGT is related to the reducing properties of GSH and the thiol metabolites that originate from GGT activity. Thiols – especially when dissociated to their thiolate anion forms ($R-S^-$) – can effect the reduction of metal cations, e.g., iron and copper. Electrons can then be transferred in turn from metal ions to molecular oxygen, thus generating the reactive oxygen species (ROS) superoxide anion and hydrogen peroxide, both capable of stimulating prooxidant reactions. The sequence – a true “redox cycling” of metals (13) – can proceed with even minimal concentrations of metal ions as long as the electron donors (thiols) and electron acceptor (molecular oxygen) are available. In this way, and paradoxically, the “reducing” abilities of thiols are eventually turned into overall “oxidizing” effects. Stark et al. (14) were the first to propose that the catabolism of GSH can play a prooxidant role under selected conditions. These authors suggested that the GGT-mediated cleavage of GSH – allegedly through the generation of the more reactive thiol cysteinyl-glycine – could cause the reduction of ferric iron Fe(III) to ferrous iron Fe(II), thus starting an iron-dependent redox-cycling process liable to result in the production of ROS and the stimulation of oxidative reactions.

Subsequent studies in our and other laboratories have repeatedly documented the production of ROS during the catabolism of GSH mediated by cellular GGT. The biochemical processes involved have been characterized (reviewed in ref. (15)), and some of the redox effects occurring at the cellular level have been described, e.g., effects on the activation status of transcription factors and on apoptotic signaling pathways (15–17).

These findings have refined our understanding of GGT function and have implicated GGT in unexpected scenarios. At a cellular level, it is conceivable that the prooxidant effects of plasma-membrane GGT activity are normally balanced by its established role in favoring the cellular uptake of precursors for GSH resynthesis, thus allowing the maintenance of cellular

antioxidant defenses. Recent epidemiological data showing that lower dietary uptake and plasma levels of some antioxidants can predict an increase in serum GGT levels (18, 19) appear to point to a similar antioxidant defensive role for serum GGT as well. On the other hand, the functions of soluble GGT activity in serum are less directly related to the cellular supply of GSH, and conditions may exist in which its prooxidant effects are predominant. In blood, the free iron required for such processes could originate from circulating iron storage proteins; in fact, it has been shown that GGT prooxidant effects can be sustained by both transferrin and ferritin (15, 20). The emerging prooxidant potential of serum GGT can help to explain recent findings, e.g., the observation that baseline serum GGT can predict future elevations of serum F_2 -isoprostanes, a sensitive marker of oxidative stress (21, 22).

The involvement of GGT in redox biochemistry does not appear, however, to characterize all of its functions. Additional redox-independent connections of GGT have recently been suggested in a study pointing to a possible (enzyme-independent) role of the protein in the regulation of bone resorption (23). Is yet another season starting for GGT significance?

The relationships of serum GGT with morbidity and mortality, including cardiovascular risk

The relationships between serum GGT levels and disease have received continuous attention for decades. Constitutive high serum GGT has been reported in families as an inherited autosomic dominant character and in the apparent absence of any disease (see e.g., (24)). Genetic effects could in principle affect either the release of GGT from liver cells or its clearance from circulation. Other liver enzymes, however, partly share these effects, and this seems to indicate that genetic factors may be operative at the level of GGT release in blood (25). The importance of genetic factors is still a matter for discussion; the current view is that a mixture of genetic and environmental factors can influence serum GGT (25, 26).

In the past, serum GGT was interpreted as a mere indicator of alcohol consumption, and its association with morbidity was initially reported from this perspective (27, 28). Conigrave et al. (29) were the first to appreciate that GGT can have a predictive value irrespective of hepatic disease or alcohol consumption. Such a role of GGT as an independent predictor of mortality from all causes has been confirmed by several subsequent studies (30–32). With respect to cardiovascular diseases, it was precociously observed that serum GGT levels can be correlated with increased risk of myocardial infarction (33–35). More recently, association of the risk of stroke with serum GGT was also observed (36, 37). These associations are partly explained by the known correlations of GGT with recognized factors in the pathogenesis of cardiovascular disorders, such as changes in blood lipids

(36, 38, 39), body mass index (39, 40), hypertension (28, 41–43), glucose intolerance (44), insulin resistance (45) and type 2 diabetes (21, 46, 47). For several of these associations a marked genetic component was observed, with only a minor dependence on environmental factors; it was suggested, therefore, that a genetic predisposition may exist to abdominal obesity, insulin resistance and other signs of the so-called “metabolic syndrome”, and that it may also include elevation of GGT and other liver enzymes (25).

It is worth emphasizing that most of the studies mentioned above refer to “normal” serum GGT, i.e., values within the laboratory reference range, which would otherwise raise no specific health concerns. Of particular interest was the study by Wannamethee et al. (35), in which attention was focused on ischemic heart disease (IHD). In a large prospective study (7613 middle-aged men, with an 11-year follow-up), GGT levels in the normal range were strongly associated with all-cause mortality, and the association was largely due to a significant increase in deaths from ischemic heart disease in the top quintile of GGT distribution. Serum GGT was positively correlated with preexisting ischemic heart disease, diabetes mellitus, antihypertensive medication, systolic and diastolic blood pressure, total and high-density lipoprotein (HDL) cholesterol, heart rate and blood glucose, while a negative association was observed with physical activity and lung function. After adjustment for these variables, elevated GGT (highest quintile, ≥ 24 U/l, vs. the remaining subjects) was still associated with a significant increase in mortality from all causes and from IHD. The increased risk of IHD mortality was more marked for patients with evidence of ischemic heart disease at screening, particularly those with previous myocardial infarction (35). The increased mortality in men without heart disease was obviously dependent on other causes; on the other hand, the findings in ischemic patients clearly pointed to a connection of GGT with underlying atherosclerotic coronary artery disease.

The envisaged connection of GGT with atherosclerotic disease was subjected to a detailed assessment in one of our own prospective studies (48). The study included a 6-year follow-up of 469 patients with ischemic syndrome and angiographically documented coronary artery disease. After correction for other cardiovascular disease risk factors (age, smoking, serum cholesterol, left ventricular ejection fraction, body mass index, diabetes mellitus) or confounding factors (serum alanine aminotransferase, self-reported alcohol consumption), the prognostic value of serum GGT activity for cardiac death and non-fatal infarction was confirmed. In particular, the significance of serum GGT was more evident in a subset of patients prone to plaque complications, i.e., characterized by the association of diffuse atherosclerosis (“multivessel disease”) and a history of previous myocardial infarction (approx. 36% of the whole population). The risk was increased using two different GGT cut-off values (25 or 40 U/l, both considered within the normal range), and the event excess was

concentrated within the first 3-year period (Figure 1). The prognostic significance of serum GGT was thus correlated with the diffusion of coronary artery disease. More interestingly, the significance of serum GGT also appeared to depend on the instability of plaque, as indicated by the fact that the prognostic value of GGT disappeared after revascularization by angioplasty (Figure 1B). This procedure is considered as a means of plaque stabilization (49). Thus, the unfavorable prognosis signaled by elevated serum GGT seems to apply specifically to patients with vulnerable plaques, suggesting that connections of some kind must exist between GGT and the processes involved in plaque instability.

Alcohol consumption is a common potentially confounding factor in these types of study. The complex issue of possible connections among the three factors – alcohol consumption, serum GGT and cardiovascular risk – was recently analyzed by Jousilahti et al. (50). In a random sample of 3666 men aged 25–74 years, prevalent ischemic heart disease (IHD) was correlated with GGT, carbohydrate-deficient transferrin (CDT, the other recognized marker of alcohol consumption) and self-reported alcohol consumption. CDT levels were inversely and GGT levels positively correlated with IHD risk. In a composite risk assessment, subjects with normal CDT (≤ 20 U/l) and elevated GGT levels (>80 U/l) had nearly eight-fold adjusted IHD risk, as compared to subjects with normal GGT and elevated CDT levels (50). As CDT is to be taken as a more reliable marker of alcohol consumption than GGT itself (51), these data seem to indicate that alcohol consumption indeed has a protective effect on cardiovascular prognosis, an effect repeatedly confirmed (52), and that the prognostic association of serum GGT must be independent of alcohol-related liver injury. It is worth considering that the latter association might stem indirectly from the known relationships of GGT with two other IHD risk factors, hypertension and insulin resistance (28, 41–43, 47).

A role for GGT in the pathogenesis of atherosclerosis?

A major portion of cardiovascular pathology is associated with the vascular damage induced by atherosclerotic degeneration, a process in which the oxidation of low-density lipoprotein (LDL) is thought to be critical. Potent catalysis of LDL oxidation can be effected by metal cations, e.g., iron, provided that electron donors are available to effect their reduction (53). Thiol compounds such as cysteine and homocysteine are known to reduce Fe(III) and promote Fe(II)-dependent LDL oxidation. In systems including ADP-Fe(III) complexes, GSH itself can reduce iron to a certain extent; the reaction rate is, however, increased significantly when GGT is also present – an effect observed over a broad range of GGT activities and GSH concentrations (54). Previous studies have shown that the reactivity of the thiol residue in cys-

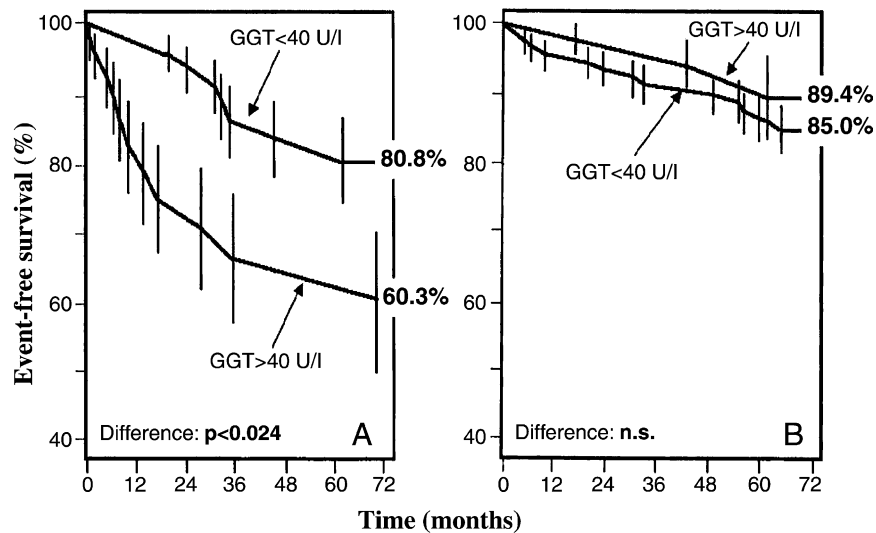


Figure 1 Event-free survival according to serum GGT activity: A: in a population of 168 patients with coronary artery disease, history of previous myocardial infarction and multiple-vessel disease (119 with serum GGT < 40 U/l and 49 with GGT > 40 U/l); and B: in a subset of the same population, having undergone revascularization by means of angioplasty. Vertical lines represent confidence intervals (data from ref. (45), modified).

teinyl-glycine – the product of GGT-mediated GSH cleavage – is significantly higher than in GSH (55). The ability of GGT to enhance reduction of Fe(III) by GSH might therefore be mediated through the formation of cysteinyl-glycine, bearing a thiol moiety which dissociates more rapidly at near-neutral pH and can thus act as a stronger Fe(III) reductant than GSH itself. Our trials confirmed that purified cysteinyl-glycine can reduce ADP-chelated Fe(III) to an extent equaling that observed with GSH plus GGT. Accordingly, purified GGT was also found to stimulate LDL oxidation in vitro, in the presence of concentrations of iron in the range of those actually detectable in atherosclerotic tissue (54). Additional experiments indicated the ability of GGT to induce the reductive delocalization of transferrin-bound iron, i.e., a physiological iron source (15).

The observations above support the view of a potential role of GGT-mediated reactions in atherogenesis. If these views are correct, then cysteinyl-glycine generated at sites of GGT activity would provide conditions liable to promote LDL oxidation and favor the progression of atherosclerotic disease. This hypothesis has received substantial support from histochemical studies, showing that intense GGT activity is present in the intimal layers of human atherosclerotic lesions, apparently expressed in CD68⁺ macrophage-derived foam cells (54, 56). Consistent with this, GGT-positive foam cells were found to co-localize with immunoreactive oxidized LDL (57). Catalytically active GGT could also be detected in microthrombi adhering to the surface of atheromas (15), a finding certainly worth further investigation.

The origin of GGT activity detectable in atherosclerotic lesions is, however, unclear. A possibility is that the expression of GGT is for some reason up-regulated in the macrophages present in the intimal space; monocytes/macrophages normally express low levels of GGT activity (58). As an alternative, the GGT activ-

ity found in atherosclerotic plaques might originate from circulating GGT, with a mechanism similar to the extravasation of (oxidized) LDL lipoprotein into the intimal space. Serum GGT has in fact been shown to be associated with several fractions of circulating lipoproteins – including Lpx – in cholestatic patients, as well as in normal subjects (59, 60). Artur et al. (61) showed that in normal subjects the apolipoprotein B (apoB)-bound GGT activity accounts for 5.5% of total serum GGT activity, and the association of GGT with LDL was demonstrated (up to approx. 30% of total serum activity) in patients with hepatocellular carcinoma (62). With respect to cardiovascular diseases, a study is currently under way in our laboratories aimed at identifying possible alterations of this GGT/LDL association in cardiac patients presenting with atherosclerotic changes of varying extents. Figure 2 shows preliminary results obtained in controls, i.e., healthy, non-atherosclerotic subjects. The fraction of GGT activity that is detectable in the plasma β -lipoprotein fraction shows a significant correlation with total serum GGT levels, over values that include the “normal” range. The completion of a comparable analysis in cardiac/atherosclerotic patients should give us clues as to whether GGT from atherosclerotic plaques can have a plasma origin or not.

The current hypotheses proposed to explain the association of serum GGT levels with prognosis of cardiovascular disorders are based on the assumption that the process must involve liver tissue at some step, as a source of the GGT found in serum. Thus, it has been proposed that a likely sequence of events might start with a condition of insulin resistance, causing the onset of fatty liver, which in turn is associated with oxidative stress and the depletion of hepatic GSH. The latter event would result in an induction of GGT, accompanied by increased release of the enzyme from the liver to the blood (47, 63). According to this view, serum GGT elevation would

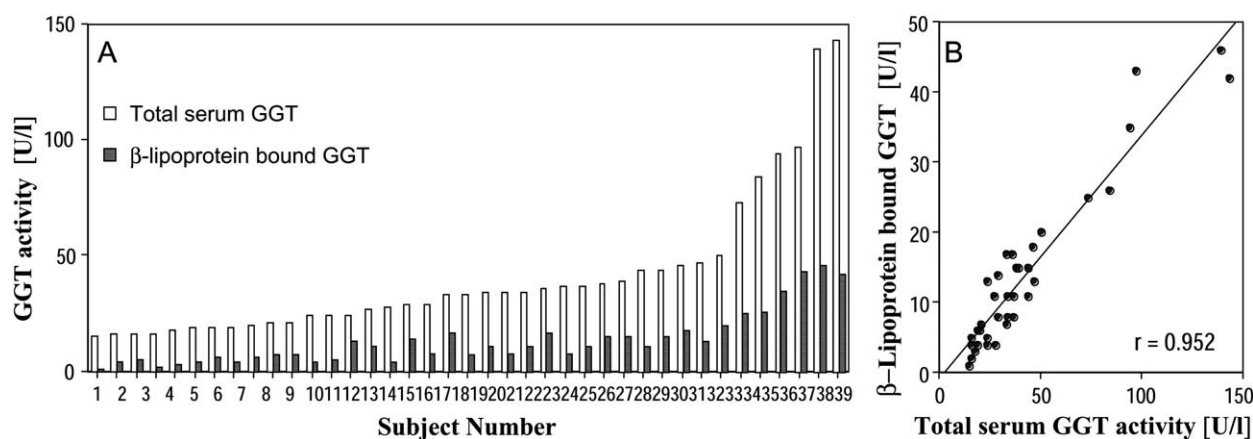


Figure 2 Association of serum GGT activity with plasma β -lipoprotein fraction in a population of 39 subjects with no signs of cardiovascular disease (age range: 43–76 years). A: Distribution of individual values in the population; B: linear correlation of lipoprotein-bound GGT levels with corresponding total serum GGT values.

simply signal the occurrence of a pathophysiological process, but not any involvement in its determination. However, on the contrary, the finding of GGT activity in vascular lesions and of the ability of GGT to mediate redox/prooxidant reactions in cooperation with metal ions might eventually lead to a direct implication of GGT activity in the atherosclerotic process, the underlying cause of cardiovascular disorders. In this novel perspective, new relevance will be gained by studies reporting the correlation of serum GGT with serum ferritin (27, 64, 65), i.e., with the status of body iron stores, which has received much attention as a potential factor in the pathogenesis of atherosclerosis. These relationships between serum GGT and ferritin, and iron in general, will form part of a prospective study that is currently under way in our laboratories.

Concluding remarks – serum GGT as a cardiovascular prognostic marker

In conclusion, with regard to ischemic heart and brain disease, serum GGT appears to have all the features of a true prognostic marker:

1. Optimal sensitivity-specificity of the diagnostic assay (63);
2. Epidemiological evidence of its presence in apparently healthy people before the occurrence of events (39, 66); and
3. Real improvement in the ability to predict subsequent events (32, 35, 48, 50).

The new insights into the role of GGT and thiols in atherosclerosis not only increase our understanding of the disease, but also have practical clinical applications in risk stratification and the targeting of therapy. Elevation of serum GGT predicts the outcomes for patients in unselected populations as well as those for patients with diagnosed IHD, thus adding to the prognostic information provided by other, traditional risk factors. Prospective studies will soon tell us how these findings relate to possible abnormalities in iron

metabolism, or to markers of low-grade chronic inflammation. Indeed, an association between serum GGT levels and C-reactive protein was described recently (21). These findings, and others possibly to come, will allow identification of the combinations at highest risk of vulnerable plaque, and then strategies can be devised for the medical stabilization of lesions, thus offering an alternative (or supplement) to percutaneous or surgical procedures.

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