

## Gamma-Glutamyltransferase, Atherosclerosis, and Cardiovascular Disease Triggering Oxidative Stress Within the Plaque

Michele Emdin, MD, PhD; Alfonso Pompella, MD, PhD; Aldo Paolicchi, MD, PhD

The serum determination of gamma-glutamyltransferase ( $\gamma$ -GT) activity is a low-cost, highly sensitive and accurate, and frequently used laboratory test. Although it is considered to be an index of hepatobiliary dysfunction and alcohol abuse,<sup>1</sup> recent epidemiology and pathology studies have suggested its independent role in the pathogenesis and clinical evolution of cardiovascular diseases brought on by atherosclerosis.<sup>1,2</sup>

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The prospective study by Ruttman and colleagues in 163 944 Austrian adults studied for 17 years shows that  $\gamma$ -GT is independently associated with cardiovascular mortality.<sup>3</sup> Serum  $\gamma$ -GT activity had a prognostic impact on fatal events of chronic forms of coronary heart disease, congestive heart failure, and ischemic or hemorrhagic stroke. This was found to be true in both sexes, with a clear dose-response relationship, and with a stronger prognostic significance of  $\gamma$ -GT in younger participants.

These findings from a large unselected cohort unequivocally confirm previous observations that  $\gamma$ -GT is associated with overall mortality and cardiovascular events, in both unselected populations<sup>4–7</sup> and patients with ascertained coronary artery disease, independent of all confounders including liver function and alcohol use.<sup>8,9</sup>

### Well-Known Versus “Unknown” $\gamma$ -Glutamyltransferase

$\gamma$ -GT is the enzyme responsible for the extracellular catabolism of glutathione (GSH,  $\gamma$ -glutamyl-cysteinyl-glycine), the main thiol intracellular antioxidant agent in mammalian cells.<sup>1</sup> It is present, linked through a small lipophilic sequence of its larger subunit, on the cell surface membrane of most cell types; although the same protein is produced in all tissues, differences in the sugar moieties allow that only the liver  $\gamma$ -GT is detectable in serum.<sup>10</sup> Most serum  $\gamma$ -GT is bound to carriers, such as  $\alpha$ - and  $\beta$ -lipoproteins and albumin.<sup>1</sup>

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From the Institute of Clinical Physiology, National Research Council (CNR), Pisa, Italy (M.E.), and the Department of Experimental Pathology, University of Pisa Medical School, Pisa, Italy (A.P., A.P.).

Correspondence to Michele Emdin, MD, PhD, Cardiovascular Medicine Dept, Institute of Clinical Physiology, National Research Council (CNR), Via Giuseppe Moruzzi 1, 56124 Pisa, Italy. E-mail emdin@ifc.cnr.it

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This association is likely to occur within hepatocytes, before  $\gamma$ -GT releases in serum, through still-unknown mechanisms.

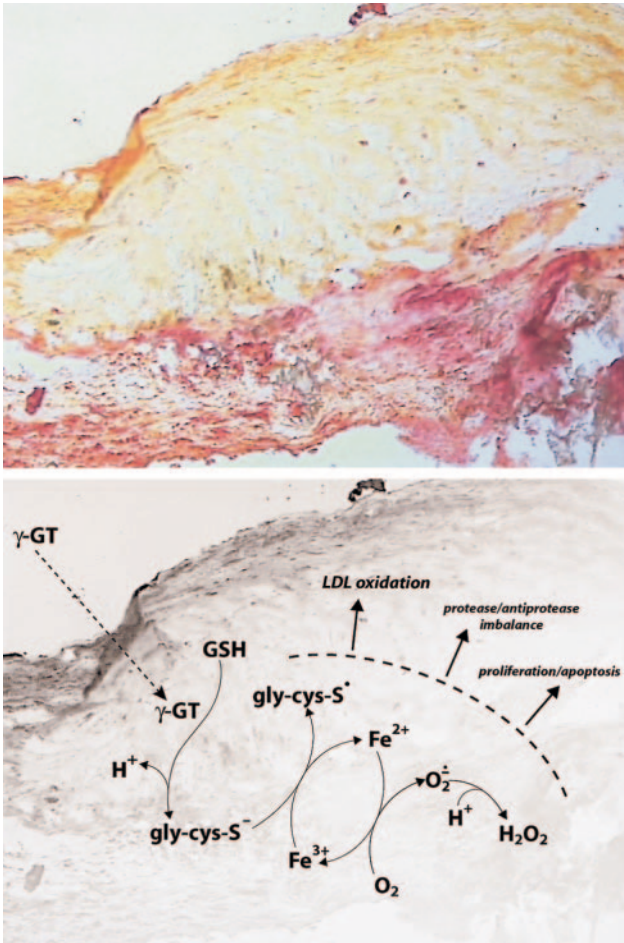
Serum  $\gamma$ -GT activity is affected by genetic and environmental factors, with heritability estimated at 0.52.<sup>11</sup> Within its normal range, it has many other determinants, even stronger than liver function or alcohol consumption.<sup>1</sup> The findings of the Austrian Vorarlberg Health Monitoring and Promotion Program,<sup>3</sup> a large unselected Norwegian population,<sup>12</sup> and a prospective study of 7613 middle-age British men<sup>6</sup> show a strong positive association between serum  $\gamma$ -GT level and body mass index, alcohol use, smoking, total lipoprotein and HDL, serum cholesterol, uric acid, serum triglycerides, heart rate, systolic and diastolic blood pressure, antihypertensive medication, preexisting ischemic heart disease, diabetes mellitus, and blood glucose use of oral contraceptives and menopause; pregnant women had lower values.<sup>3,5,6,12</sup>  $\gamma$ -GT showed a negative association in men in regard to physical activity and lung function (forced expiratory volume in 1 second) and coffee consumption.<sup>3,5,6</sup>

### Prooxidant Effects of Glutathione Hydrolysis by $\gamma$ -Glutamyltransferase

Catalytically active  $\gamma$ -GT has been found within atherosclerotic cerebral, carotid, and coronary plaques from autopsic studies and surgical endoarterectomy, colocalized with oxidized density lipoproteins (LDL) and CD68<sup>+</sup> foam cells.<sup>13–15</sup> As concerns the possible association between  $\gamma$ -GT and inflammatory process, it should also be considered that  $\gamma$ -GT has a key role in the interconversion of the glutathione-containing inflammatory mediator leukotriene C4 into leukotriene D4.<sup>16</sup>

Although the exact mechanism leading to accumulation of  $\gamma$ -GT within the plaque is unknown, the association of  $\gamma$ -GT to lipoproteins suggests that LDL lipoproteins can carry  $\gamma$ -GT activity inside the plaque,<sup>17</sup> where free iron is also present.<sup>18</sup> In the extracellular milieu,  $\gamma$ -GT is the only enzyme responsible for GSH catabolism by hydrolysis of its  $\gamma$ -glutamyl bond between glutamate and cysteine. This reaction produces cysteinyl-glycine moieties, which are usually taken within intracellular milieu by the action of membrane dipeptidases, as precursors for GSH resynthesis.<sup>1</sup>

Cysteinyl-glycine is a powerful reducer of Fe<sup>3+</sup> in the extracellular milieu—and likely at the plaque level—that is able to simultaneously generate Fe<sup>2+</sup> and a free thyl radical; subsequent reactions lead to the formation of superoxide anion radical and hydrogen peroxide.<sup>2</sup> These  $\gamma$ -GT-mediated reactions have been shown to catalyze the oxidation of LDL lipoproteins,<sup>13</sup> likely contributing to oxidative events influ-



A, Histochemical demonstration of  $\gamma$ -GT enzyme activity within a frozen section of coronary atheroma from endoarteriectomy in vivo. Histochemical reaction for  $\gamma$ -GT enzyme activity was performed with the specific substrate  $\gamma$ -glutamyl-4-methoxy-2-naphthylamide and the diazonium salt Fast Garnet GBC as a chromogen. A strong  $\gamma$ -GT activity (red stain) is selectively present in correspondence of the core of the atheroma, whereas the fibrous cap stains negative (original magnification  $\times 20$ ).

Adapted with permission from Paolicchi et al.<sup>15</sup> B,  $\gamma$ -GT metabolism of glutathione (GSH) within the plaque. The hydrolysis of GSH originates cysteinyl-glycine, which is a powerful reductant of  $\text{Fe}^{3+}$ , able to simultaneously generate  $\text{Fe}^{2+}$  and a free thiyl radical. Thereafter, oxygen reactive species, by the same reaction, contribute to a prooxidant effect, leading to LDL oxidation and likely contributing to other processes, such as metalloproteinase activation, cell proliferation, and apoptosis.

encing plaque evolution and rupture (Figure, A and B). Because of the iron dependence of  $\gamma$ -GT mediated reactions, the described association between increased body iron stores and excess risk of acute myocardial infarction suggests that iron metabolism could influence the predictive value of serum  $\gamma$ -GT.<sup>19</sup> The oxidative stress mediated by  $\gamma$ -GT could thus play a relevant role in the evolution of atherosclerotic plaque and its instabilization: apoptosis of cellular elements of the lesion, plaque erosion and rupture, enhanced platelet aggregation, and thrombosis.<sup>20</sup>

### $\gamma$ -GT from Bench to Bedside

Ruttmann and colleagues established the prognostic value of  $\gamma$ -GT for cardiovascular events at serum levels that lie within

normal values. The receiver operating characteristics analysis suggested  $\gamma$ -GT cutoff values of 15.5 U/L for men and 10.5 U/L for women, corresponding to 27.6 U/L and 18.7 U/L, respectively, for measurements made at 37°C.<sup>3</sup> An increasing number of population studies have evaluated the relationship between serum  $\gamma$ -GT activity and mortality, since the observation of Conigrave et al in 1993<sup>4</sup> that indicated the predictive value of  $\gamma$ -GT for mortality, irrespective of hepatic disease or alcohol consumption.

Thereafter, Wannamethee confirmed the negative prognostic value of  $\gamma$ -GT<sup>6</sup>: After adjustment for all confounders, elevated  $\gamma$ -GT (highest quintile  $\geq 24$  U/L versus the rest) was associated with a significant increase in mortality from all causes and from ischemic heart disease, namely in individuals with a history of ischemic heart disease. This suggested a link with underlying atherosclerotic coronary artery disease. Another more recent study aimed at evaluating the long-term prognosis among 714 patients with a small or unconfirmed acute myocardial infarction indicated  $\gamma$ -GT as an independent risk factor for death in association with age, previous myocardial infarction, smoking, and glucose.<sup>9</sup>

The link with underlying atherosclerotic coronary artery disease has been demonstrated by a prospective study of our group. During a 6-year follow-up of 469 patients with ischemic syndrome and angiographically documented coronary artery disease,<sup>8</sup> and after correcting for other cardiovascular risk factors (eg, age, smoking habit, serum cholesterol, left ventricular ejection fraction, body mass index, diabetes mellitus) or confounding factors, such as serum alanine-aminotransferase level and self-reported alcohol consumption, the prognostic value of serum  $\gamma$ -GT activity for cardiac death and nonfatal infarction has been confirmed in a subset of patients corresponding to 36% of the whole population, characterized by the association of multivessel disease and history of myocardial infarction. The risk increased with different cutoff values of 25 and 40 U/L, however, within the normal range, and the event excess was concentrated within the first 3-year period.<sup>8</sup> The prognostic significance of  $\gamma$ -GT seems thus correlated not only with the extent of coronary artery disease but also with the instability of the plaque.

As concerns stroke mortality, the finding by Ruttmann's group confirm earlier reports from a large Finnish study in unselected populations in both sexes, which demonstrated that  $\gamma$ -GT is associated with an independent prognostic value for ischemic stroke, independent of self-reported alcohol consumption, whereas another common marker of alcohol consumption, carbohydrate-deficient transferrin, was inversely associated with risk.<sup>3,7</sup>

Ruttmann's group does not report any information about the diagnosis of cardiovascular disease. This makes it impossible to understand whether the prognostic role of  $\gamma$ -GT is associated either with evolution or final complications of the atherosclerotic process.<sup>3</sup> History of previous ischemic heart disease, in particular of previous myocardial infarction, regardless of ventricular function, strengthened  $\gamma$ -GT predictive value, indicating that considering coronary patients as a whole, those with higher  $\gamma$ -GT activity may constitute a subset at highest risk of repetitive events.<sup>4,8</sup> The extent of coronary atherosclerotic disease enhances the  $\gamma$ -GT prognos-

tic significance, which may act on a larger substratum.<sup>8</sup> Interestingly, both percutaneous and surgical revascularization were able to abolish the  $\gamma$ -GT prognostic value, confirming its intrinsic link with the evolution of the plaque.<sup>8</sup>

The pathogenetic mechanism proposed for the role of  $\gamma$ -GT in promoting the atherosclerotic process should be considered independent, complementary, and synergistic to conventional determinants. In fact, level of serum  $\gamma$ -GT is significantly genetically determined;<sup>6</sup> serum  $\gamma$ -GT activity holds an independent prognostic value within reference level range in all epidemiological studies after adjustment for confounders such as indicators of hepatic function and alcohol consumption (the latter has often been found to exert a rather protective effect);<sup>3–8,13</sup>  $\gamma$ -GT maintains its predictive value after adjustment for other established cardiovascular risk factors, which, however, affect at least in part its concentration, such as obesity, smoking, total serum cholesterol, arterial hypertension, diabetes, reduced physical activity, with particular emphasis in patients with history of preexisting ischemic heart disease.<sup>3–8,13</sup>

### Do We Need Another Risk Factor?

In conclusion, the recent insights into the role of thiol metabolism in atherosclerosis not only increase our understanding of the disease but also have practical clinical applications in risk stratification and targeting of therapy for a clinical challenge of growing worldwide importance. Elevation in serum  $\gamma$ -GT activity predicts outcomes in unscreened populations and in patients with ascertained ischemic heart disease, independently of myocardial damage, thus adding to prognostic information provided by traditional risk factors.

As for ischemic cerebral and heart disease,  $\gamma$ -GT serum assay seems to have all of the main features of a true prognostic marker: the diagnostic assay has optimal sensitivity-specificity,<sup>1,2</sup> epidemiological evidence of its presence before the event in apparently healthy people and patients with clinical overt disease increases our ability to predict it,<sup>3,5–9,13</sup> it has additive and independent predictive value in comparison with established risk factors.<sup>3,5–9</sup> Finally, as concerns ischemic heart disease, the prognostic impact of  $\gamma$ -GT can be abolished by therapeutic interventions, such as coronary revascularization.<sup>8</sup>

The physiological function of  $\gamma$ -GT enzyme activity as a source of peptide precursors for intracellular GSH resynthesis, as well as the current clinical concept of its serum activity as the consequence of a compensatory overexpression in response to hepatobiliary dysfunction or alcohol toxic effect, is challenged. The evidence is growing in favor of a detrimental role, triggering a prooxidant action within the atherosclerotic plaque. Additional investigation would permit the identification among subjects with higher  $\gamma$ -GT value those with a higher risk of developing clinical disease, allowing definition of the interrelationships with iron metabolism alterations, markers of inflammatory process, of glucose and metabolic disease, and with presence, features, and extent of atherosclerotic vessel disease, to better define the most risky combination for the vulnerable plaque and the best medical

strategies for the stabilization of lesions, rather than percutaneous or surgical procedures.

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