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Additive prognostic value of gamma-glutamyltransferase in coronary artery disease

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Abstract

Background: Serum gamma-glutamyltransferase activity (GGT) has been documented as an independent cardiovascular risk factor. However, to-date its value has not been compared with C-reactive protein (CRP) and other indexes in a multimarker prognostic strategy in patients with coronary artery disease.
Methods: We prospectively evaluated 474 subjects with angiographically documented CAD. GGT and traditional humoral and clinical parameters were measured at hospital admission. A multivariate model was used to predict all-cause and cardiac mortality.
Results: GGT showed an independent prognostic value after adjustment for possible confounders, including alcohol consumption, and beyond established risk factors, such as extent of coronary atherosclerotic disease, left ventricular ejection fraction, age, serum glucose, cholesterol subfractions, and C-reactive protein (CRP). At a 3-year follow-up, cardiac mortality was 9% in patients with serum GGT activity >25 U/L vs. 3.5% in those with serum GGT<25 U/L ($p=0.028$). The association of three independent biomarkers (higher GGT, CRP, fasting glucose) identified a subgroup of 45 patients with the highest risk of cardiac death at 3 years (26.6%, vs. no event or 2.7% in the subsets of 87 and 198 patients with, respectively, no/one risk factor above cut-off value, $p<0.0001$).
Conclusions: GGT is confirmed as independent risk factor in patients with established coronary artery disease. GGT, CRP, fasting glucose show an additive prognostic value, whereas low values of these biomarkers identify a subset of patients with the lowest risk of cardiac death.

Keywords: Gamma-glutamyltransferase; GGT; C-reactive protein; CRP; Prognosis; Coronary artery disease

1. Introduction

The serum determination of gamma-glutamyltransferase (GGT) activity is a low-cost, highly sensitive and accurate laboratory test frequently used as an index of hepato-biliary dysfunction and alcohol abuse [1]. Recent pathology studies [2,3] have suggested the independent role of GGT in the pathogenesis of cardiovascular diseases brought by atherosclerosis: its activity has been observed in coronary atherosclerotic plaques, colocalized with low density lipoprotein (LDL) and foam cells [2]. Moreover, it has been shown that glutathione hydrolysis by GGT can trigger iron-catalysed LDL oxidation [3], as well as production of reactive oxygen species [3], likely promoting plaque complications.

Epidemiology studies on large unselected populations, including Framingham's one, have suggested an independent value of serum GGT activity in the prognostic evaluation of cardiovascular diseases [4–8]. In particular, findings of a very large epidemiological Austrian study including data over 17 years (1985–2001) from 163,944 volunteers of the "Vorarlberg Health Monitoring and Promotion Program" [5]

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confirmed the prognostic value of serum GGT activity on fatal events in chronic forms of coronary heart disease, congestive heart failure, and ischemic or hemorrhagic stroke. More recently, Duk-Hee Lee and co-workers showed an independent predictive value of serum GGT activity for non-fatal myocardial infarction and fatal coronary heart disease in a large (28,838 middle-aged men and women) unselected cohort: the prognostic role is stronger among subjects aged <60 and subjects with type 2 diabetes [8].

As concerns patients with proven coronary artery disease a prognostic value of serum GGT activity for cardiac mortality and myocardial infarction was shown in a previous study of our group, after adjustment for other cardiac risk factors and alcohol consumption, but not for C-reactive protein level and subfractions of cholesterol [9]. These biomarkers are associated with different specific pathogenetic aspects of atherosclerosis process (from inflammation to lipid accumulation and oxidation within the plaque) [10]: thus, for predicting cardiovascular risk a multimarker approach, using a composite of several biomarkers measured in parallel is desirable [10].

Therefore, in patients with angiographically ascertained coronary artery disease over a 3-year follow-up, we assessed the putative prognostic value of GGT, its relationship with established risk factors, for the first time including C-reactive protein (CRP) and cholesterol subfractions, in order to select the best multimarker risk combination.

2. Methods

2.1. Patients

We prospectively evaluated 474 consecutive adult patients undergoing coronary arteriography, for diagnostic work-up of ischemic heart disease between September 1997 and September 2000 at the CNR Institute of Clinical Physiology in Pisa, Italy, who showed atherosclerotic coronary artery lesions diagnosed by coronary angiography; patients undergoing angiography without significant atherosclerotic involvement of coronary vessels were excluded from the study.

The research protocol has been approved by our institutional ethics committee and the subjects gave informed consent; the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

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 by blood pressure >140 mmHg for systolic and >85 mmHg for diastolic value in more than one determination or by treatment with antihypertensive drugs), hypercholesterolemia (defined by plasma cholesterol level of >200 mg/dL or by treatment with hypolipemic drugs), obesity (body mass index >30), diabetes mellitus (defined by either antidiabetic therapy or a fasting glucose level of >126 mg/dL in more than one determination), and non-cardiovascular diseases. Serum total cholesterol, high density lipoprotein (HDL) and LDL cholesterol fraction (levels used to identify abnormal HDL, LDL cholesterol were 35 and 150 mg/dL, respectively), triglycerides, glucose, GGT, CRP, alanine-aminotransferase (ALAT) and aspartate-aminotransferase (ASAT), transferrin, and serum iron levels were recorded, body mass index was determined, and arterial pressure was obtained according to WHO guidelines.

All coronary angiograms were evaluated by cardiologists unaware of the patients' risk factor profile. Coronary artery disease was defined as the presence of significant stenosis of at least 50 percent 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.

2.2. Follow-up

Follow-up started at hospital admission and continued until study termination (September 2001): independent interviewers obtained information directly from patients, relatives, or Institute cardiologists or general practitioners, regarding the date of occurrence of cardiac death or revascularization procedure, between the time of angiography and September 2001. Information about time and cause of death was obtained from death certificates, post-mortem reports and family doctors. The end-point considered was cardiac death; patients who died from non-cardiac causes or following revascularization procedures were considered withdrawn alive. No patient was lost to follow-up. Patients initially treated medically were included in the follow-up until time of revascularization procedure; their subsequent follow-up was censored (withdrawn alive). Deaths were classified as due to cardiac disease when caused by acute myocardial infarction, sudden death or congestive heart failure, and cardiac mortality was adopted as the end-point of the study. The classification of cause of cardiac vs. non-cardiac death was blinded to risk factors evaluation.

2.3. Biochemical measurements

Serum GGT activity and all other hematochemical data were obtained within a same day from antecubital vein blood samples after overnight fasting, according to the usual clinical laboratory procedures. In particular, GGT was assayed at 37 °C using L-gamma-glutamyl-3-carboxy-4-nitroanilide as substrate, by a Beckman CX7 automatic analyser, as all other biomarkers, except for high sensitivity CRP (Abbott TDX analyser, Abbott-USA).

2.4. Statistical analysis

Due to the skewness of the serum GGT values distribution, a natural logarithmic transformation was applied for statistical analysis when required. The same was applied for other markers, when needed. Values are presented as mean ± standard deviation (SD).

Survival curves were analysed using the Kaplan–Meier estimate. The comparison between survival curves was performed using the log-rank and the generalised Wilcoxon tests. In addition, to identify which prognostic variables were significant their individual effect on survival was evaluated with the Cox proportional hazards model [11]. According to a stepwise selection process, variables were entered or removed from the regression equation on the basis of a computed significance probability ('maximised partial-likelihood ratio') [11]. This allowed the identification of a subset of variables, all having significant independent correlation with the incidence of cardiac death. We considered the median value of the distribution, as cut-off point for the serum values of GGT (25 U/L), CRP (0.8 mg/dL), fasting glucose (118 mg/dL) for cardiac mortality as end-point. Median values of serum GGT and glucose coincided with the best cut-off points obtained by means of parametric Receiver Operating Characteristic (ROC) analysis, which furnished the value of 2.5 mg/dL as cut-off point for CRP [12].

The analysis was performed on the following continuous variables: age, body mass index, systolic and diastolic arterial pressure. Other variables were considered as dichotomous for multivariate analysis: GGT activity, cholesterol, HDL and LDL cholesterol, CRP level, triglycerides, glucose, sex, family history of ischemic heart disease, history of previous myocardial infarction, diabetes mellitus, hypercholesterolemia, arterial hypertension, smoking habit, alcohol consumption, number of diseased vessels (i.e. single- vs. multiple vessel disease), and left ventricular ejection fraction.

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 hazard ratios are presented with 95% confidence intervals and all p values are two-sided. The P-spline function was used to fit a general spline term within the Cox model; GGT activity, cholesterol, HDL and LDL cholesterol, CRP level, triglycerides, glucose were considered as continuous variables for this kind of analysis [13]. Probability values were considered significant when <0.05.

Table 1
Characteristics of the population studied.

	All	Males	Females	M vs. F
	(n=474)	(n=381)	(n=93)	p
Age, years	64±11	63±11	69±9	0.000
BMI, kg/m^2	27.3±4.1	27.3±4.4	27.4±4.0	0.714
Diseased vessel, n	1.8±.9	1.8±.9	1.8±.8	0.950
LVEF, %	53±12.0	52.±12.0	54±11	0.352
GGT, U/L	35±36	36±34	29±44	0.000
Total chol., mg/dL	196±45	192±42	208±54	0.003
Trigl., mg/dL	129±92	131±85	122±117	0.149
LDL chol., mg/dL	128±41	135±51	127±38	0.087
HDL chol., mg/dL	42±12	40±11	49±13	0.000
% SAT	24±11	25±11	21±9	0.009
Hb, g/dL	13.8±1.6	14.1±1.5	12.8±1.5	0.000
CRP, mg/dL	4.8±21.2	5.4±23.6	2.5±3.8	0.367
Ferritin, ng/mL	180±157	195±166	119±91	0.000
Glucose, mg/dL	100±35	98±32	107±44	0.045
S-iron, mcg/dL	53±12	52±12	54±11	0.352
Fibrinogen, mg/dL	343±113	339±112	357±115	0.190
ESR, mm/h	24±20	22±19	33±23	0.000
Creatinine, mg/dL	1.1±.4	1.2±.4	0.9±0.2	0.000

BMI: body mass index; LVEF: left ventricular ejection fraction; GGT: gamma-glutamyltransferase; Chol.: cholesterol; Trigl.: triglycerides; LDL: low density lipoprotein, HDL: high density lipoprotein; % SAT: saturation of transferrin; Hb: hemoglobin; CRP: C-reactive protein; S-iron: serum iron; ESR: erythrocyte sedimentation rate.

Table 2
Independent predictors at univariate and multivariate analysis of cardiac mortality.

	Hazard ratio	95% C.I.	p value
Univariate analysis			
Age (per year)	1.07	1.02–1.12	0.001
S-iron	2.23	1.97–5.14	0.049
Transferrin	2.41	1.08–2.62	0.020
GGT	2.45	1.06–5.62	0.020
Previous MI	2.64	1.15–5.22	0.017
Diseased vessel n	3.05	1.22–7.59	0.009
LDL	3.20	1.34–7.61	0.017
Glucose	3.93	1.75–8.83	0.006
CRP	5.75	1.99–16.68	0.001
LVEF	7.26	2.91–18.08	0.000
Multivariate analysis			
Age (per year)	1.06	1.02–1.12	0.001
GGT	2.44	1.04–5.65	0.028
Diseased vessel n	2.55	1.01–6.45	0.009
Glucose	3.47	1.38–7.00	0.004
CRP	5.98	2.04–17.51	0.001
LVEF	7.31	2.99–18.01	0.000

MI: myocardial infarction, GGT: gamma-glutamyltransferase, S-Iron: serum iron, LDL: low density lipoprotein, CRP: C-reactive protein, LVEF: left ventricular ejection fraction.

3. Results

3.1. Characteristics of patients

The mean age of the 474 patients with ischemic heart disease in our study (381 males and 93 females) was 64±10 years (range 33–88, 63±11 and 69±9, for males and females, respectively); 223 (47%) had a diagnosis of previous myocardial infarction (see main clinical and biohumoral features in Table 1). Diabetes and arterial hypertension were diagnosed in 183 (39%) and 178 (28%) patients, respectively; a total of 164 patients were smokers (35%), 255 were drinkers (59%), with an average daily alcohol intake of 30±17 g. Aspirin was used in 431 patients (91%), calcium-channel blockers in 322 (68%), nitrates in 379 (80%), beta-blockers in 237 (50%), and angiotensin-converting-enzyme inhibitors in

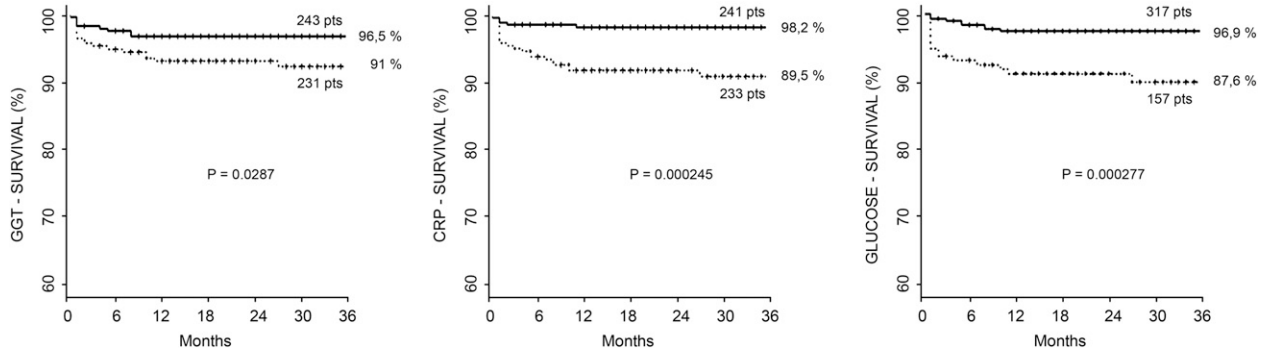

Fig. 1. Left panel: survival after 3 years of follow-up, according to serum gamma-glutamyltransferase (GGT) activity among 474 patients with coronary artery disease (243 with GGT < 25 U/L and 231 with GGT > 25 U/L). Center panel: event free survival, according to serum C-reactive protein (CRP) level (241 with CRP < 0.8 mg/dL and 233 with CRP > 0.8 mg/dL). Right panel: survival according to serum glucose level (317 patients with glucose < 118 mg/dL and 157 with glucose > 118 mg/dL).

156 (33%), and statins in 204 (43%). At coronary angiography 220, 146 and 77 patients had one-, two- or three-vessel disease (46%, 31%, 16%, respectively); 31 patients (7%) had left main-stem artery disease. No significant correlation between the extent of atherosclerosis (number of vessels involved) and baseline GGT value. Mean left ventricular ejection fraction was 52.6 ± 12.0%. Percutaneous transluminal coronary angioplasty was performed in 279 patients (59%) during the follow-up, while 62 (13%) underwent coronary artery bypass grafting. The distribution of serum GGT in the 474 patients with coronary artery disease was skewed to the right, with a mean value of 34.6 ± 36.0 U/L.

3.2. Identification of risk factors for cardiac death

Considering withdrawal at first cardiac event, non-cardiac death or revascularization procedure, a median follow-up of 18 months (range 4–1080 days) was achieved in the study population. During the follow-up period, there were 26 cardiac deaths: 3 sudden deaths, 6 due to acute myocardial infarction and 17 due to congestive heart failure. A total of 14 deaths were excluded from the analysis: 9 were due to non-cardiac diseases, 5 were related to cardiac surgery.

On the whole, at the univariate analysis we identified the following significant predictors of cardiac death (Table 2): plasma levels of CRP, age, fasting glucose, extent of coronary artery disease (i.e. number of vessels diseased), serum GGT, left ventricular ejection fraction, serum iron, history of previous myocardial infarction, serum transferrin, LDL cholesterol. At the multivariate analysis, plasma levels of CRP, age, fasting glucose, extent of coronary artery disease (i.e.: number of vessels diseased), GGT, left ventricular ejection fraction were the only independent predictors. Alcohol consumption and serum ALAT had no prognostic value in this population. The same variables resulted as independent predictors, also when serum cholesterol, glucose, and arterial pressure were considered as continuous variables, and when dichotomized evaluation of history of hypercholesterolemia, diabetes, and arterial hypertension was taken into account.

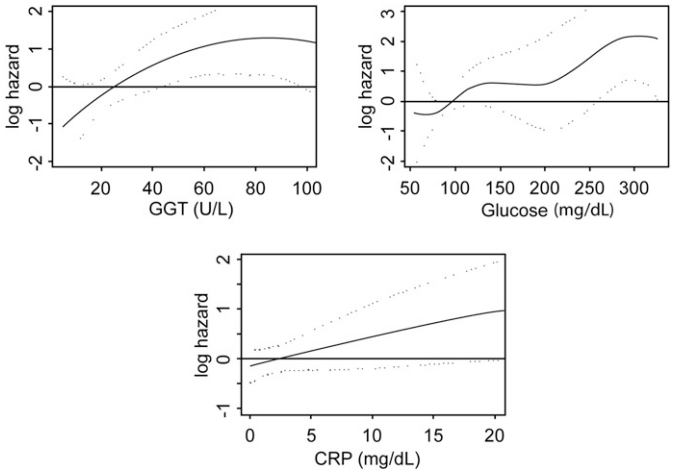

Fig. 2. P-spline analysis (the lines reflect the 95% confidence intervals) of the association of biomarkers with the risk of cardiac death shows a dose–response relationship for serum gamma-glutamyltransferase (GGT) activity (top left), glucose level (top right), C-reactive protein (CRP) level (bottom).

3.3. Prognostic significance of serum GGT activity, CRP, glucose

At 3 years Kaplan–Meier estimate of mortality was 9.0%, for patients with GGT activity above 25 U/L, and 3.5% for those with levels below 25 U/L ($p=0.028$). Among recognised cardiovascular risk factors, serum GGT activity showed a weak correlation only with triglycerides ($R=0.142$, $p=0.05$) and with fasting glucose ($R=0.174$, $p=0.04$). Moreover, GGT correlated with serum ALAT level ($R=0.347$, $p=0.0001$) and, though marginally, with alcohol consumption ($R=0.128$, $p=0.05$).

The 3-year estimate of mortality was 10.5% for patients with CRP activity above 0.8 mg/dL, and 1.8% for those with levels below 0.8 mg/dL ($p=0.0001$), while it was 12.2%, for patients with blood glucose above 118 mg/dL and 3.1% for those with levels below 118 mg/dL ($p=0.0006$). Event-free patient survival is shown in Fig. 1 for each of the three biomarkers.

P-spline analysis of the association of biomarkers with the risk of cardiac death, adjusted for other multivariable covariates, showed a dose–response relationship. Serum GGT activity prognostic impact started at 25 U/L level with a plateau around 50 U/L; serum glucose level showed a relevant impact on risk above 100 mg/dL, with a second steep increase above 200 mg/dL value; serum CRP level related risk increased progressively (Fig. 2).

3.4. Combined predictive value of biomarkers

As shown in Fig. 3, the association of the three biomarkers (higher GGT, CRP, blood glucose) identified a subgroup of 45 patients with the highest risk of cardiac death (26.6%) at 3 years (hazard ratio 29.21, CI: 23.99–35.58). On the contrary, there was no event among the 87 patients with lower values of GGT, CRP, glucose, and only 2.7% of deaths in the subset of 198 patients with positivity for only one biomarker. As shown in Fig. 3, the group of 144 patients, with elevation of two factors showed an intermediate level of risk (cardiac mortality 8.2%), which was significantly different from other groups.

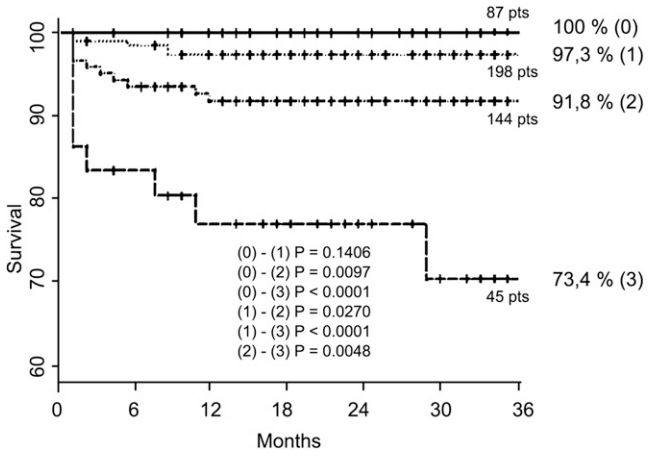

Fig. 3. Survival among 474 patients with coronary artery disease, according to: (0) the absence of risk factors (RF) above cut-off value (see text) among C-reactive protein, gamma-glutamyltransferase, glucose, (1) the elevation of one RF, (2) combination of two RF above cut-off value, (3) combination of three RF above cut-off value. Vertical lines represent confidence intervals. Mortality was absent, after 3-year follow-up, among patients with no elevated marker, whereas the association of three RF increased the risk of cardiac death up to 26.6%.

4. Discussion

Our findings confirm the independent mid-term (three-years) prognostic value of serum GGT activity in patients with coronary artery disease, after adjustment for confounders (including ALAT levels and alcohol consumption) and established cardiovascular risk factors, and show, for the first time in patients with ascertained coronary artery disease, its additive value to other established biomarkers, such as CRP, and serum glucose.

Though severity and extent of coronary atherosclerotic involvement are major determinants of cardiac events, other factors may favor plaque complications leading to life-threatening events [14]. Thus, the combined evaluation of biohumoral marker, reflecting different pathogenetic mechanisms triggering plaque destabilization might improve risk stratification [10,15]. In particular, glucose and CRP evaluation may be linked to the glycation of critical protein targets [16] and to a low-grade inflammation [17] within the plaque, respectively.

Lee et al. have recently confirmed the predictive value of GGT among diabetic subjects, from a cohort of 28,838 Finnish men and women aged 25–74 years [8], supporting the hypothesis that the pathogenetic mechanism involving GGT may be additive to those specifically promoting the atherosclerotic process in diabetes (e.g. lipoprotein glycation).

A specific pathogenetic mechanism driven by GGT likely concurs to the instabilization of the plaque [18]: an increasing epidemiological evidence points out the possible role of oxidant reactions mediated by GGT in pathological processes leading to myocardial infarction, cardiac death, and ischemic stroke [4–9], suggesting a general pathway to plaque instabilization. GGT is the main determinant of extracellular hydrolysis of glutathione [1], releasing the dipeptide cysteinyl-glycine. The reactive thiol of cysteinyl-glycine may cause the reduction of ferric Fe(III) to ferrous iron Fe(II), starting a redox-cycling process with subsequent production of the reactive oxygen species superoxide anion and hydrogen peroxide [1]. The prooxidant effects of GGT are likely to occur within atherosclerotic coronary plaque where the catalytically active enzyme has been histochemically identified in correspondence of oxidised lipoproteins and $CD68^{+}$ macrophagic foam cells [2,3]. Serum GGT is partially adsorbed onto lipoproteins [19], which could carry GGT inside the atherosclerotic lesion, where iron is present within the plaque gruel at sufficient concentrations to sustain GGT prooxidant reactions [20].

Serum GGT activity showed a best prognostic cut-off value of 25 U/L, confirming previous findings [9]. Moreover, there was a graded response relationship, with risk increasing with GGT values up to 50 U/L, as shown by P-spline analysis. In vitro observations have shown that GGT, in presence of iron ions, catalyses LDL oxidation [3]: the range of effective GGT concentration was 25–100 U/L [3], the same associated with cardiac risk in the present study.

Serum GGT activity improves the predictive accuracy in a multimarker approach including several known predictors of the disease: our findings show that 18% of the cohort showed no risk factor above cut-off value, among CRP, GGT, glucose and were eventless, whereas 42% of patients, showing only one biomarker higher than cut-off value, had a low (2.7%) cardiac mortality.

On the other hand, 9% of patients with a combination of the three risk factors, showed the highest risk (26.6% of events), while the remaining 30% with only two risk factors showed intermediate risk, indicating their additive value.

As a study limitation, its design does not allow to derive significant information on the drug influence on biomarker prognostic value, due to the lack of serial measurements. Moreover, since the time of the study pharmacological treatment has been substantially changed, especially with regard to the use of statins, whose effect on GGT, however, is still unknown.

In conclusion, GGT serum activity, a marker of metabolic and cardiovascular risk in unselected populations [5,6,21,22], as well as in patients with suspicion of coronary acute syndrome [23], is confirmed as an independent cardiac risk factor even in ischemic patients with established coronary atherosclerosis, demonstrating an additive prognostic value for cardiac death in comparison with glucose and CRP. Moreover, GGT, CRP, and glucose below the cut-off level, or a higher value of any one biomarker characterize a high percentage of patients who show a smallest risk of future events.

References

[1] Whitfield JB. Gamma glutamyl transferase. Crit Rev Clin Lab Sci 2001;38:263–355.

[2] Paolicchi A, Emdin M, Ghliozeni E, et al. Human atherosclerotic plaques contain gamma-glutamyl transpeptidase enzyme activity. Circulation 2004;109:1440.

[3] Paolicchi A, Minotti G, Tonarelli P, et al. Gamma-glutamyltranspeptidase-dependent iron reduction and LDL oxidation: a potential mechanism in atherosclerosis. J Invest Med 1999;47:151–60.

[4] Wannamethee G, Ebrahim S, Shaper G. Gamma-glutamyltransferase: determinants and association with mortality from ischemic heart disease and all-causes. Am J Epidemiol 1995;142:699–708.

[5] Ruttmann E, Brant LJ, Concin H, Diem G, Rapp K, Hanno Ulmer H. Gamma-glutamyl transferase as a risk factor for cardiovascular disease mortality: an epidemiologic investigation in a cohort of 163,944 Austrian adults. Circulation 2005;112:2130–7.

[6] Lee DS, Evans JC, Robins SJ, et al. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk. The Framingham Heart Study. Arterioscler Thromb Vasc Biol 2007;27:127–33.

[7] Meisinger C, Doring A, Schneider A, Lowel H, for the KORA Study Group. Serum gamma-glutamyltransferase is a predictor of incident coronary events in apparently healthy men from the general population. Atherosclerosis 2006;189:297–302.

[8] Lee DH, Silventoinen K, Hu G, et al. Serum gamma-glutamyltransferase predicts non-fatal myocardial infarction and fatal coronary heart disease among 28 838 middle-aged men and women. Eur Heart J 2006;27:2170–6.

[9] Emdin M, Passino C, Michelassi C, et al. Prognostic value of serum gamma-glutamyl transferase activity after myocardial infarction. Eur Heart J 2001;22:1802–7.

[10] Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation 2006;113:2335–62.

[11] Dixon WJ, Brown MB, Engelman L, Jennrich RI. BMDP Statistical Software Manual. Berkeley: University of California Press; 1990.

[12] Greiner M. Two-graph receiver operating characteristic (TG-ROC): update version supports optimization of cut-off values that minimise overall misclassification costs. J Immunol Methods 1996;191:93–4.

[13] Eilers PH, Marx BD. Flexible smoothing with P-splines and penalties. Stat Sci 1996;11:89–121.

[14] Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation 2005;111:3481–8.

[15] Jaffe AS, Babuin L, Apple FS. Biomarkers in acute cardiac disease: the present and the future. J Am Coll Cardiol 2006;48:1–11.

[16] Cipollone F, Iezzi A, Fazia M, et al. The receptor RAGE as a progression factor amplifying arachidonate-dependent inflammatory and proteolytic response in human atherosclerotic plaques: role of glycemic control. Circulation 2003;108:1070–7.

[17] Norja S, Nuutila L, Karhunen PJ, Goebeler S. C-reactive protein in vulnerable coronary plaques. J Clin Pathol 2007;60:545–8.

[18] Emdin M, Pompella A, Paolicchi A. Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: triggering oxidative stress within the plaque. Circulation 2005;112:2078–80.

[19] Paolicchi A, Emdin M, Passino C, et al. Beta-lipoprotein- and LDL-associated serum gamma-glutamyltransferase in patients with coronary atherosclerosis. Atherosclerosis 2006;186:80–5.

[20] Pang JH, Jiang MJ, Chen YL, et al. Increased ferritin gene expression in atherosclerotic lesions. J Clin Invest 1996;97:2204–12.

[21] Whitfield JB. Serum gamma-glutamyltransferase and risk of disease. Clin Chem 2007;53:1–2.

[22] Grundy SM. Gamma-glutamyl transferase: another biomarker for metabolic syndrome and cardiovascular risk. Arterioscler Thromb Vasc Biol 2007;27:127–33.

[23] Karlson BW, Wiklund O, Hallgren P, Sjölin M, Lindqvist J, Herlitz J. Ten-year mortality amongst patients with a very small or unconfirmed acute myocardial infarction in relation to clinical history, metabolic screening and signs of myocardial ischaemia. J Intern Med 2000;247:449–56.