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FULL RESEARCH PAPER

Big gamma-glutamyltransferase is associated with epicardial fat volume and cardiovascular outcome in the general population

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Aims Gamma-glutamyltransferase (GGT) has been recognized as a cardiovascular risk factor, and its highest molecular weight fraction [big GGT (b-GGT)] is found in vulnerable atherosclerotic plaques. We explored the relationship between b-GGT, computed tomography findings, and long-term outcomes in the general population.

Methods and results Between May 2010 and October 2011, subjects aged 45–75 years living in a Tuscan city and without known cardiac disease were screened. The primary endpoint was a composite of cardiovascular death or acute coronary syndrome requiring urgent coronary revascularization. Gamma-glutamyltransferase fractions were available in 898 subjects [median age 65 years (25th–75th percentile 55–70), 46% men]. Median plasma GGT was 20 IU (15–29), and b-GGT was 2.28 (1.28–4.17). Coronary artery calcium (CAC) score values were 0 (0–60), and the volume of proatherogenic epicardial fat was 155 mL (114–204). In a model including age, sex, low-density lipoprotein (LDL) cholesterol, current or previous smoking status, hypertension, diabetes, obesity, b-GGT independently predicted epicardial fat volume (EFV) ($r = 0.162$, $P < 0.001$), but not CAC ($P = 0.198$). Over a 10.3-year follow-up (9.6–10.8), 27 subjects (3%) experienced the primary endpoint. We evaluated couples of variables including b-GGT and a cardiovascular risk factor, CAC or EFV. Big GGT yielded independent prognostic significance from age, LDL cholesterol, current or previous smoking status, hypertension, diabetes, obesity, but not CAC or EFV. Conversely, GGT predicted the primary endpoint even independently from CAC and EFV.

Conclusion Big GGT seemed at least as predictive as the commonly available GGT assay; therefore, the need for b-GGT rather than GGT measurement should be carefully examined.

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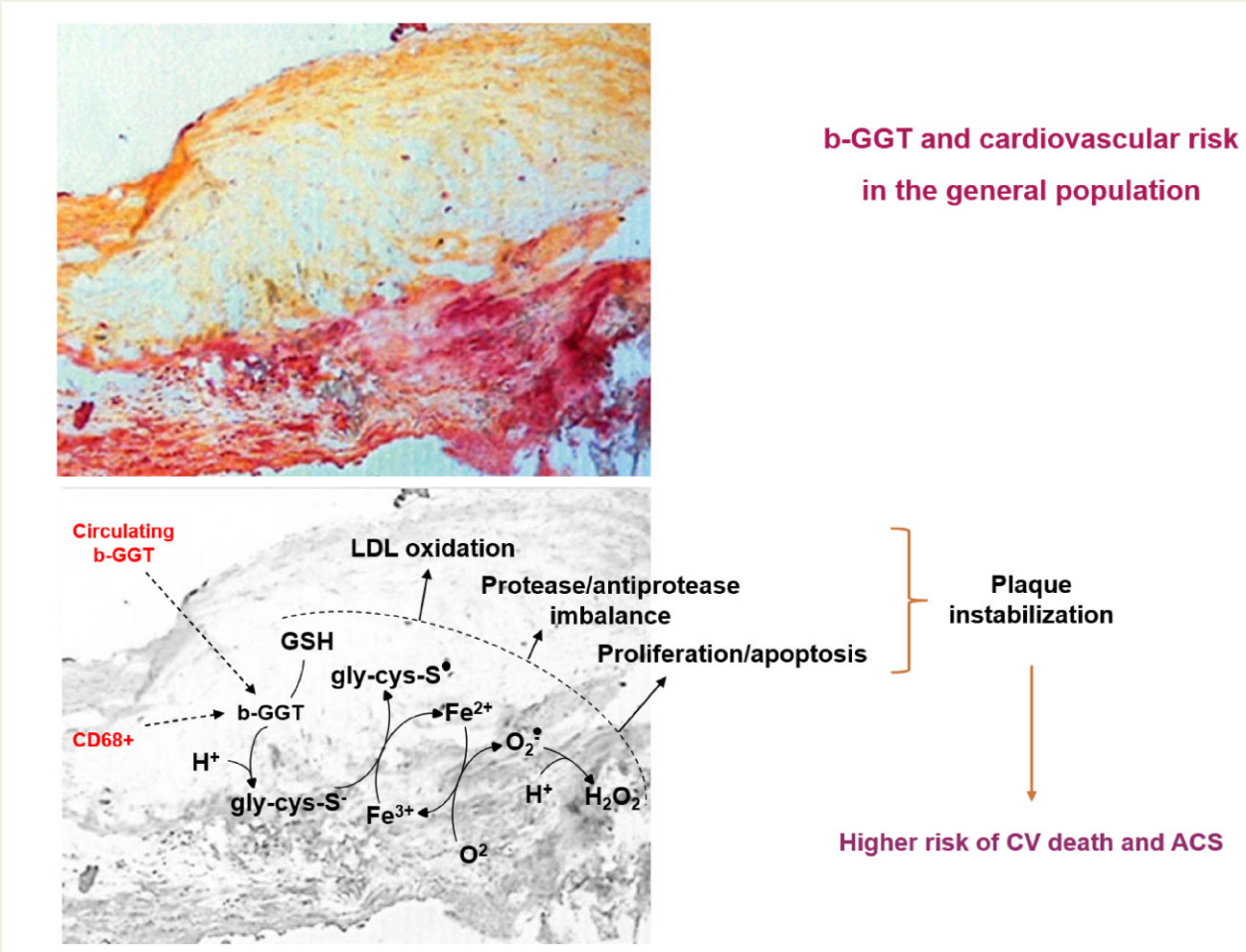

Graphical Abstract Big gamma-glutamyltransferase (b-GGT) and cardiovascular risk in the general population. b-GGT deriving from the bloodstream or released by macrophages (CD68+ cells) promotes oxidative stress within the plaque. This ultimately leads to plaque destabilization. ACS, acute coronary syndrome; CV, cardiovascular. Modified with permission from Emdin *et al.*, 2005.[26]

Keywords GGT • Primary prevention • Cardiovascular risk • Atherosclerotic plaques • Coronary artery calcium • Epicardial fat

Introduction

Coronary artery disease (CAD) is the first cause of mortality in industrialized countries and often manifests with myocardial infarction (MI) or cardiac death.[1] The risk of cardiovascular events in asymptomatic individuals is generally evaluated through prediction models relying on the integrated assessment of traditional risk factors, such as age, gender, systolic blood pressure, and smoking status in the European Society of Cardiology (ESC) Score charts.[2] Nevertheless, these risk factors have been estimated to predict just up to 65% of events.[3] This justifies the search for additional elements for prognostic stratification, including laboratory biomarkers or imaging indicators.

Among the latter, the extent of coronary artery calcium (CAC) by computed tomography (CT) has been proposed as a risk modifier,[2] given its additive value over established cardiovascular risk factors.[4,5] The CAC score correlates with the coronary plaque burden[6] but is not an indicator of plaque inflammation and instability.[7] Furthermore, the amount of adipose tissue within the pericardium might provide a link between metabolic dysregulation and vascular wall inflammation,[8,9] since the epicardial fat releases cytokines with a paracrine pro-atherogenic effect on the coronary arteries.[10,11] Indeed, epicardial fat volume (EFV) is associated with cardiovascular risk factors,[12] coronary calcifications,[13,14] and is predictive of significant coronary artery stenoses,[15] and major acute cardiac events.[16]

Gamma-glutamyl transferase (GGT) is an enzyme located on the external surface of membranes of all cells except for erythrocytes.[17] Serum GGT activity is associated with body mass index, alcohol intake, smoking habit, cholesterol and triglyceride levels, hypertension, and diabetes. Nevertheless, higher GGT levels, even within the reference range, predict cardiovascular risk regardless of the conventional cardiovascular risk factors and alcohol consumption, and beyond current risk charts.[18–23] Under this respect, GGT has been proposed as a risk factor for plaque destabilization.[24] Indeed, GGT catalyzes the extracellular oxidation of glutathione, the main intracellular antioxidant in mammals; the cysteinyl-glycine residue from glutathione hydrolysis then triggers the iron-dependent production of free radicals and induces lipoprotein oxidation.[25] Gamma-glutamyltransferase has been found within atherosclerotic plaques, where it likely promotes the oxidation of low-density lipoproteins (LDLs).[24,26]

Four fractions of GGT have been identified in the plasma: big-GGT (b-GGT, MW >2000 kDa), medium-GGT (m-GGT, MW 940 kDa), small-GGT (s-GGT, MW 200 kDa), and free-GGT (f-GGT, MW 70 kDa).[27–29] Circulating b-GGT levels correlate with several cardiovascular risk factors, including body mass index, systolic blood pressure, LDL cholesterol, and high-sensitivity C-reactive protein.[30] Big GGT is carried by membrane vesicles similar to exosomes,[31] and only this fraction has been identified into the atherosclerotic plaque.[32] Big GGT levels into the plaque correlate with the plasma b-GGT and macrophage infiltrates, which are a histologic marker of plaque vulnerability.[33]

In this study, we investigated for the first time whether plasma b-GGT predicts the extent of CAC and EFV, as an emerging marker of plaque instability, as well as cardiovascular outcomes, in a cohort of subjects from the general population with a 10-year follow-up.

Methods

Study cohort

The present study was carried out among participants of the Montignoso Heart and Lung Project. Montignoso is a small city in Tuscany, Italy, with about 10 000 inhabitants. Between May 2010 and October 2011, all subjects aged between 45 and 75 years were invited to participate to a population screening. Among respondents ($n = 1672$, 52% of those invited), we enrolled subjects free from known cardiac disease and without symptoms of cardiovascular disease (such as angina or intermittent claudication), without known pulmonary disorders and no evidence of active neoplasia, a life expectancy longer than 1 year, and able to express informed consent. The final study population included 1382 subjects. A team composed of a cardiologist and a pneumologist interviewed these patients using a standardized form focused on prior evidence of cardiac or lung disease and cardiovascular risk factors, as previously diagnosed. The risk of cardiovascular events was estimated using the Framingham Risk Score for Hard Coronary Heart Disease.[34] Study participants also underwent a non-contrast CT chest scan. This analysis was performed on the subset of patients with quantified total GGT and GGT fractions ($n = 898$, 65%). The study conformed to the Helsinki declaration and was approved by the Institutional Review board. All patients gave written informed consent.

Computed tomography scan

All patients underwent a low-dose (120 KV, 60 mA) CT scan by a 64 detectors scanner (Aquilion 64, Toshiba Medical Systems, Otawara, Japan). Prospective electrocardiographic triggering (set at 50% of the expected next RR interval) in sequential slice mode was used, with 0.5 mm collimation thickness and 0.5 mm thick transverse images. The total estimated dosimetry was 2–3 mSv. The CAC score was calculated through the Agatston method using a densitometric program (Vitrea 2.0, Vital Images Inc., Minnetonka, MN, USA). Coronary artery disease severity was graded based on CAC score values: 0, no evidence of CAD; 1–10, minimal CAD; 11–100, mild CAD; 101–400, moderate CAD; and >400, severe CAD.[35] The EFV was measured using a semi-automatic software. Cardiac annotations were realized placing point along the heart border on the central axial slice and generating a closed line, interpolated with a spline function of order 3. The contour extracted was then automatically reproduced on the other axial slices to include the heart, if present, in the delimited region of interest (ROI). The shape of the final generated cardiac contours could be manually modified if some portions of the heart were excluded by the segmentation, translating the ROI or creating new points. The references about the epicardial fat were finally represented by all the pixels contained in the cardiac ROI and with Hounsfield Unit values between –190 and –30.[36] The CT scans were read by experienced operators blinded to all other subject information.

Laboratory evaluation

Blood samples were drawn in the morning after overnight fasting. The operators who analysed the blood samples were blinded to all other patient data. Low-density lipoprotein cholesterol was estimated using the Friedwald formula, which could be used in all cases as no subject had triglyceride levels ≥400 mg/dL.[37] Analysis of total and fractional GGT activity was performed on plasma samples using a fast protein-liquid chromatography system (AKTA purifier, GE Healthcare Europe, Milan, Italy) fitted out with a gel-filtration column (Superose 6 HR 10/300 GL; GE Healthcare Europe) and a fluorescent detector (Jasco FP-2020; Jasco Europe, Lecco, Italy). Before injection, plasma samples were filtered through a 0.45 μm PVDF filter (Millipore), and each injection was carried out with 0.02 mL of filtered sample. Separation of GGT fractions was obtained by gel-filtration chromatography and the enzymatic activity was determined by post-column injection of the fluorescent substrate specific for the GGT, gamma-glutamyl-7-amido-4-methylcoumarin (γGluAMC). The enzymatic reaction, in the presence of γGluAMC and glycylglycine as acceptor of transpeptidation reaction, proceeds for 4.5 min in a reaction coil (PFA, 2.6 mL) kept at the 37°C in a water bath. The AMC signal was detected by the fluorescence detector operating at excitation/emission wavelengths of 380/440 nm; the intensity of the fluorescence signal was expressed in arbitrary fluorescence units. Under these reaction conditions, the area under the curve is proportional to total GGT activity, while the area under each single peak is proportional to the corresponding GGT fraction activity. The detection limit of the method is 0.167 U/L and the determination limit is 0.5 U/L; within-day and between-day coefficient of variations are 3% and 2%, respectively. About 50 min was necessary to evaluate the enzyme activity in a single sample. A computer programme (Version 7 MATLAB MathWorks, Inc.) was used to quantify total and fractional GGT activity and to resolve the overlap of the resulting chromatograms. The sum of fractional GGT activity represents on average the 99% of total GGT activity. As stated above, the following GGT fractions were identified: b-GGT (MW >2000 kDa), m-GGT (MW 940 kDa), s-GGT (MW 200 kDa), and f-GGT (MW 70 kDa).[27,30]

Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology collaboration (CKD-EPI) formula.[32] N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured through the ECLIA assay (Elecsys 2010 analyser, Roche Diagnostics, Basel, Switzerland).

Follow-up

Study participants were managed according to contemporary guidelines for primary cardiovascular prevention.[2,33] In February 2021, information on all-cause mortality was retrieved from healthcare administrative records. These data were integrated with institutional health records and phone calls to patients, parents, or general practitioners. The attribution of cardiovascular death required documentation of significant arrhythmias or cardiac arrest, or death attributable to heart failure, MI or pulmonary embolism in the absence of any other precipitating factor. The primary endpoint was a composite of cardiovascular death or acute coronary syndrome (ACS) requiring urgent coronary revascularization; it could be ascertained in 888 patients out of 898 (99%) because of missing data on the cause of death in 10 patients. The secondary endpoints were: cardiovascular death or any coronary revascularization (i.e. either elective or urgent); cardiovascular death; and all-cause death.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (version 22, 2013) and R (http://www.r-project.org/, version 3.2.3, 2015). All patients had baseline clinical, laboratory, and CT data. Normal distribution was evaluated through the Shapiro–Wilk test. As all variables had a non-normal distribution, they were reported as median and 25th–75th percentile. Mean differences among groups were evaluated through the Kruskal–Wallis one-way analysis of variance. Discrete variables were compared by the χ^2 test with Yates correction. The strength of correlations between couples of variables was quantified through the Spearman's rho index. Multivariate linear regression analysis was performed to search for predictors of ln-transformed CAC and EFV; the model included b-GGT or total GGT (both ln-transformed) and several cardiovascular risk factors (age, sex, LDL cholesterol, current or previous smoking status, hypertension, diabetes, and obesity). Cubic spline interpolation was carried out to represent the changes in risk according to biomarker values; five knots were considered. The biomarker value for which the relative hazard ratio (HR) = 1 was chosen as the value corresponding to the inflection point of the curve, above which the slope of the curve becomes steeper. Predictors of the primary and secondary endpoints were searched among baseline variables through bivariate Cox regression analysis (to account for the small number of events, in agreement with the 'one in ten' rule). Multicollinearity was searched by calculating the variance inflation factor, with a conservative threshold of 3. *P*-values <0.05 were considered as significant.

Results

Population characteristics

Study participants ($n = 898$, 65%) displayed limited differences from the other individuals evaluated as part of the Montignoso project ($n = 484$, 35%; Supplementary material online, *Table S1*). Study participants had a median age of 65 years (25th–75th percentile: 55–70), and 46% of them were males. They had normal left ventricular ejection fraction [60% (55–60)] and preserved renal function [eGFR 91 mL/min/1.73 m^2 (79–100)]; 41% had hypertension, 27% obesity, 11% diabetes, and 32% were current or previous smokers. Ferritin was 89 µg/L (50–155), high-sensitivity C-reactive protein 0.2 mg/dL (0.1–0.4), and NT-proBNP 60 ng/L (35–109). Furthermore, LDL cholesterol was 130 mg/dL (108–154), and triglycerides 92 mg/dL (67–130).

Median plasma GGT was 20 IU (15–29), and b-GGT was 2.28 (1.28–4.17). A moderate degree of correlation between ln-transformed values of GGT and b-GGT was observed (rho 0.763, $P < 0.001$; Supplementary material online, *Table S2*). Coronary artery calcium score values were 0 (0–60), with 51% of subjects having CAC = 0, 11% having CAC 100–399, and 9% having CAC ≥400. Finally, EFV was 155 mL (114–204) (*Table 1*). Among patients without obesity or diabetes ($n = 595$, 66%), the correlation between total and

Table 1 Patient characteristics

	All patients ($n = 898$)
Age (years)	65 (55–70)
Men, *n* (%)	416 (46)
BMI (kg/m^2)	27 (25–31)
Obesity, *n* (%)	240 (27)
eGFR (mL/min/1.73 m^2)	91 (79–100)
CKD stages 3/4/5, *n* (%)	38/2/0 (4/0/0)
Haemoglobin (g/dL)	14 (13–15)
Ferritin (µg/L)	89 (50–155)
LVEF (%)	60 (55–60)
NT-proBNP (ng/L)	60 (35–109)
Hypertension, *n* (%)	369 (41)
Fasting glycaemia (mg/dL)	100 (92–111)
Diabetes, *n* (%)	100 (11)
Current smoker, *n* (%)	133 (15)
Previous smoker, *n* (%)	246 (27)
hs-CRP (mg/dL)	0.2 (0.1–0.4)
Total cholesterol (mg/dL)	214 (187–242)
LDL cholesterol (mg/dL)	130 (108–154)
HDL cholesterol (mg/dL)	60 (50–71)
TG (mg/dL)	92 (67–130)
AST (IU/L)	21 (18–25)
ALT (IU/L)	21 (16–27)
ALP (IU/L)	55 (46–67)
Total bilirubin (mg/dL)	0.8 (0.6–1.0)
Direct bilirubin (mg/dL)	0.1 (0.1–0.1)
Aspirin, *n* (%)	73 (8)
Statin, *n* (%)	118 (13)
GGT (IU/L)	20 (15–29)
b-GGT (IU/L)	2.28 (1.28–4.17)
m-GGT (IU/L)	0.56 (0.24–1.07)
s-GGT (IU/L)	5.54 (3.42–10.78)
f-GGT (IU/L)	10.58 (8.46–13.71)
CAC score	0 (0–60)
CAC = 0	460 (51)
CAC 1–99	258 (29)
CAC 100–399	101 (11)
CAC ≥400	79 (9)
EFV (mL)	155 (114–204)

Values are given as median (25th–75th percentile) or absolute *n* (%). Gamma glutamyltransferase (GGT) fractions of plasma activity: b-GGT, big; f-GGT, free; m-GGT, medium; s-GGT, small.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAC, coronary artery calcium; EFV, epicardial fat volume; HDL, high-density lipoproteins; LDL, low-density lipoproteins; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TG, triglycerides.

Table 2 Big-gamma-glutamyltransferase (b-GGT), epicardial fat volume (EFV), and coronary artery calcium (CAC): multivariable linear regression analysis

	Variable predicted: EFV		Variable predicted: CAC	
	Exp(B)	*P*-value	Exp(B)	*P*-value
b-GGT	0.162	<0.001	—	0.198
Age	0.247	<0.001	0.408	
Male sex	0.276	<0.001	0.266	<0.001
LDL cholesterol	—	0.465	—	0.085
Current or previous smoker	—	0.708	0.074	0.028
Hypertension	0.091	0.015	0.090	0.010
Diabetes	—	0.103	0.112	0.001
Obesity	0.335	<0.001	—	0.777

b-GGT, EFV, and CAC values were ln-transformed.
LDL, low-density lipoprotein.

b-GGT was virtually identical (rho 0.793, $P < 0.001$), the distribution of CAC values was similar (CAC = 0, 54%; CAC 100–399, 10%; CAC ≥400, 8%), and EFV was 134 mL (104–183).

Big gamma-glutamyltransferase and computed tomography findings

Ln(b-GGT) independently predicted ln(EFV) in a model including age, sex, LDL cholesterol, current or previous smoking status, hypertension, diabetes, and obesity ($r = 0.162$, $P < 0.001$), while it did not independently predict ln(CAC) ($P = 0.198$; *Table 2*). Similar results were found for total GGT (Supplementary material online, *Table S3*).

Big gamma-glutamyltransferase and outcome

Over a 10.3-year follow-up (9.6–10.8), 27 subjects out of 888 (3%) experienced the primary endpoint (cardiovascular death or ACS), 36 out of 888 (4%) died for cardiovascular causes or underwent coronary revascularization, 16 out of 888 (2%) died for cardiovascular causes, and 74 out of 898 (8%) died for any cause. The risk of all endpoints increased quite steeply with b-GGT values, with cut-off values for the different endpoints ranging around 3 (*Figure 1*). The risk increased in parallel with GGT as well (Supplementary material online, *Figure S1*).

We then evaluated couples of variables including b-GGT and a cardiovascular risk factor, CAC or EFV. For the prediction of the primary endpoint, b-GGT yielded independent prognostic significance from age, LDL cholesterol, current or previous smoking status, hypertension, diabetes, and obesity, but not CAC or EFV (*Table 3*). Conversely, GGT predicted the primary endpoint even independently from CAC and EFV (Supplementary material online, *Table S4*).

Discussion

Both epicardial fat and plaque b-GGT have been associated with plaque inflammation and risk of acute plaque changes. We report for the first time that plasma b-GGT predicts the amount of epicardial fat and displays a relationship with the risk of cardiovascular death or ACS, also independently from many cardiovascular risk factors, although not from the extent of coronary artery calcifications. Big GGT and total GGT are correlated and show a broadly similar predictive value for CT findings and outcomes.

The CAC score has emerged as a widely available, consistent, and reproducible means of assessing risk of atherosclerotic cardiovascular disease in asymptomatic people with no known CAD for planning primary prevention interventions such as statins and aspirin.[38,39] Several studies have demonstrated CAC efficiency in predicting cardiovascular events beyond traditional risk factors.[40–44] A higher CAC score correlates with a major plaque burden[6,45] and a higher risk,[46] although it is not useful to assess plaque inflammation and vulnerability,[47,48] as it is likely associated with the plaque evolution towards the stability with a fibrocalcific cap formation.[49]

The epicardial fat physiologically has biochemical, mechanical, and thermogenic cardioprotective properties,[50] although genetic, epigenetic, and environmental factors may drive the shift towards a dysfunctional phenotype characterized by a pro-inflammatory and pro-fibrotic phenotype[51] contributing to the progression of atherosclerotic plaques. Therefore, the epicardial fat has recently emerged as a novel cardiovascular risk marker. A greater EFV predicts the risk of CAD[15,52,53] and has been associated with CAD risk factors[13,54] and with plaque size and composition,[11] a relationship between EFV and different features of plaque vulnerability, such as thin-cap of the fibroatheromas and high percentage of necrosis has been demonstrated.[51] Sahasrabuddhe *et al.*[55] investigated epicardial gene expression in a case–control study of patients with CAD undergoing coronary artery bypass surgery and patients operated for heart valve disease confirming histologically the findings from previous studies that have investigated the association between dysfunctional epicardial fat secretome and the risk of CAD. Intensive statin treatment has been described to reduce EFV independently of the degree of lipid lowering, suggesting that the anti-inflammatory pleiotropic activities of statins might have a role in reducing plaque vulnerability also through a reduction in EFV. In a large meta-analysis 41 534 subjects, the authors demonstrated that in low-to-intermediate cardiovascular risk subjects, EFV was independently associated with coronary artery stenosis, myocardial ischaemia, and major adverse cardiac events.

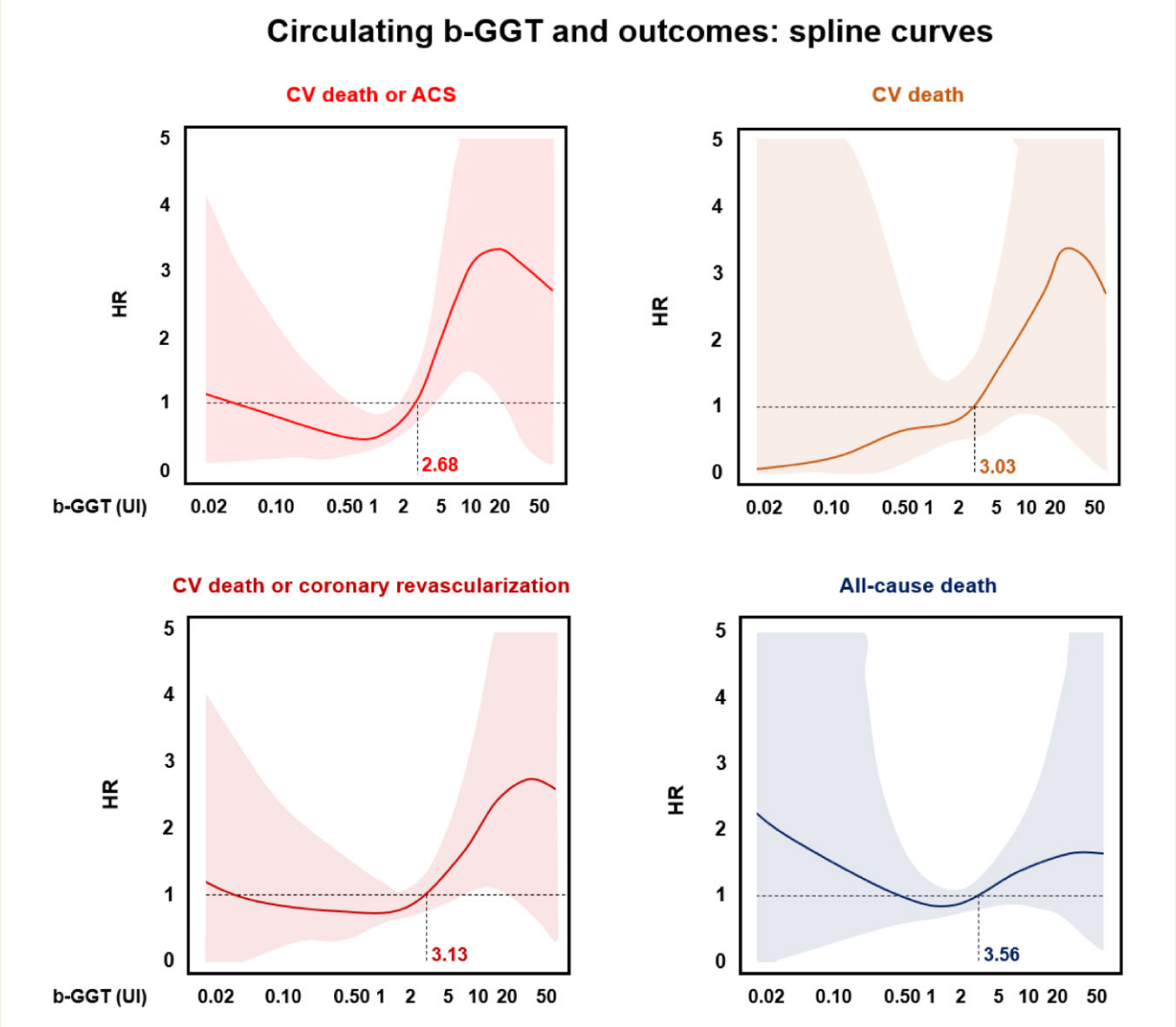

Figure 1 Big gamma-glutamyltransferase and outcome: spline curve analysis. The relationship between big gamma-glutamyltransferase and outcomes is represented through spline curves, which report the risk of outcomes according to circulating big gamma-glutamyltransferase levels. The inflection points of the curves, i.e. the big gamma-glutamyltransferase values above which the risk increases more steeply, are specified. ACS, acute coronary syndrome; CV, cardiovascular.

The authors concluded that EFV and CAC can be both imaging biomarkers of CAD, and consequently, they are associated with each other in crude analysis but probably refer to different and complementary aspects of the disease.[56]

Numerous studies confirmed the role of GGT as a cardiovascular risk factor, as discussed above. Big GGT is the only GGT fraction found in plaques, and plaque b-GGT correlated with plaque cholesterol content and plasma b-GGT fraction.[32] Moreover, higher b-GGT activity was found in thin-cap fibroatheromas and this correlated with histologic indices of plaque instability such as larger necrotic zone, higher cholesterol content, and greater macrophage infiltration.[32,33] In our cohort, circulating b-GGT (as well as total GGT) predicted EFV, which is a marker and possibly a causative factor of plaque inflammation. In turn, plaque inflammation increases the risk of acute plaque changes, manifesting as ACS.

The evidence of a relationship between b-GGT and cardiovascular outcomes support the existence of an interplay between plasma b-GGT activity, plaque inflammation and risk of ACS resulting from plaque destabilization. Big GGT has some potential to become a novel tool to refine risk prediction in the general population, beyond traditional cardiovascular risk factors,[2] allowing a more tailored management that may include the start of an antiplatelet therapy, lower LDL cholesterol targets, or a closer follow-up. Nonetheless, some crucial aspects remain to be clarified. First, GGT or b-GGT measurement

Table 3 Big-gamma-glutamyltransferase (b-GGT) for outcome prediction: bivariate Cox regression analysis

	CV death or ACS ($n = 27$)		CV death or coronary revascularization ($n = 36$)		CV death ($n = 16$)		All-cause death ($n = 74$)	
	P-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)
b-GGT	0.029	1.57 (1.05–2.35)	0.092	—	0.022	1.83 (1.09–3.06)	0.145	—
Age	0.835	—	0.030	1.05 (1.01–1.10)	0.014	1.10 (1.02–1.19)	<0.001	1.11 (1.07–1.15)
b-GGT	0.059	—	0.351	—	0.086	—	0.909	—
Male sex	0.112	—	0.022	2.26 (1.12–4.55)	0.545	—	0.056	—
b-GGT	0.038	1.55 (1.02–2.34)	0.218	—	0.059	—	0.431	—
LDL cholesterol	0.257	—	0.254	—	0.941	—	0.010	0.99 (0.98–1.00)
b-GGT	0.039	1.53 (1.02–2.28)	0.219	—	0.076	—	0.882	—
Current or previous smoker	0.253	—	0.386	—	0.581	—	0.009	1.85 (1.16–2.93)
b-GGT	0.032	1.55 (1.04–2.33)	0.280	—	0.087	—		
Hypertension	0.851	—	0.052	—	0.293	—		
b-GGT	0.032	1.54 (1.04–2.29)	0.246	—	0.089	—	0.808	—
Diabetes	0.436	—	0.046	2.23 (1.01–4.92)	0.110	—	<0.001	2.60 (1.52–4.42)
b-GGT	0.001	2.13 (1.35–3.35)	0.009	1.70 (1.14–2.53)	0.042	1.84 (1.02–3.33)	0.966	—
Obesity	0.176	—	0.210	—	0.072	—	0.075	—
b-GGT	0.086	—	0.457	—	0.093	—	0.954	—
CAC	0.002	1.30 (1.10–1.55)	<0.001	1.49 (1.28–1.74)	0.053	—	<0.001	1.24 (1.13–1.36)
b-GGT	0.168	—	0.804	—	0.150	—	0.475	—
EFV	0.071	—	0.023	3.13 (1.18–8.33)	0.321	—	0.537	—

Values of b-GGT, coronary artery calcium (CAC), and epicardial fat volume (EFV) were ln-transformed.
ACS, acute coronary syndrome; CI, confidence interval; CV, cardiovascular; HR, hazard ratio; LDL, low-density lipoprotein.

could be particularly useful in a subgroup of individuals from the general population, but our cohort was too small to allow reliable subgroup analyses. Second, the present study focused specifically on b-GGT, but this last seemed at least as predictive as the commonly available GGT assay; therefore, the need for b-GGT rather than GGT measurement should be carefully examined. Third, the cost-effectiveness of adding b-GGT or GGT measurement to the standard evaluation of cardiovascular risk should be formally evaluated, although reliable estimates of cost-effectiveness of these strategies are challenging.[2] Another possible perspective for future research is the assessment of dynamic changes of b-GGT or GGT over time and also in response to interventions such as intensive lipid lowering or smoking cessation, as well as the prognostic impact of changes in GGT or b-GGT.

In addition to these points, several limitations of this hypothesis-generating study must be acknowledged. We evaluated a relatively small cohort, albeit by far the largest cohort with GGT fractions dosed. The number of events over a 10-year follow-up was limited, reflecting a good prognosis of subjects from this general population. The small number of events obliged us to perform multiple bivariate analyses to meet the 'one in ten' rule, with a risk of false positives due to multiple comparisons; further analyses on the prognostic value of b-GGT and GGT are then warranted. GGT values were available from 65% of subjects from the whole cohort because of organizational issues, but a selection bias can be excluded because of the random nature of these issues. Gamma-glutamyltransferase and CT findings were evaluated at a single timepoint, therefore their evolution over time could not be assessed.

In conclusion, we report for the first time that plasma b-GGT activity, a putative index of plaque vulnerability, is associated with the volume of pro-atherogenic epicardial fat and holds some prognostic significance for cardiovascular death or ACS in the general population.

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology* online.

Conflict of interest: none declared.

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