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Aortic valve disease and gamma-glutamyltransferase: Accumulation in tissue and relationships with calcific degeneration

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ABSTRACT

Objective: Degenerative aortic valve disease is characterized by some of the histological features of atherosclerotic lesions. Gamma-glutamyltransferase (GGT) has been recently implicated in pathogenesis of atherosclerosis, as well as in modulation of cells involved in calcium metabolism. We aimed to evaluate the possible implication of this enzyme activity in aortic valve disease.
Methods: GGT immunohistochemistry was performed on valve leaflets of 64 patients with aortic valve stenosis undergoing valve replacement. Fractional GGT activity in plasma and tissue was analysed in a subgroup of cases by molecular exclusion chromatography.
Results: A close association was found between tissue extracellular GGT staining and lipid deposits ($p < 0.0001$). GGT was expressed by CD68-positive cells around neovessels, as well as by MMP-9- and TRAP-positive multinucleated cells in the vicinity of bone metaplasia areas. Total plasma GGT levels were associated with low HDL-c ($p = 0.028$) and high triglycerides ($p = 0.017$). Total GGT activity in tissue was negatively correlated with the extent of valves calcification ($p = 0.03$). Both serum and tissue GGT levels were negatively associated with severity of valve stenosis, as judged by peak transvalvular pressure gradients ($p < 0.0003$ and $p < 0.002$, respectively).
Conclusions: Accumulation of GGT activity inside the lipid component of valves leaflets suggests a common mechanism of lesion shaping underlying both atherosclerosis and degenerative aortic valve disease. Moreover, the finding of GGT expression in cells with an osteoclast-like phenotype, and its negative correlation with both valves calcification and degree of valvular stenosis lend additional support to the recently envisaged involvement of GGT in the homeostasis of calcified tissues.

1. Introduction

Calcified aortic valve stenosis (AS), a common valve disease in elderly, is the first cause for surgical valve replacement in United States and Europe [1]. AS is not a passive degenerative disease, but an active cellular process which shares clinical and histological similarities with atherosclerosis, including activated inflammatory cells, lipid deposits, calcified nodules and bone tissue [2–5]. Moreover, it is associated with diabetes, hypercholesterolemia, hypertension, smoking, male sex, elevated LDL-c levels, and metabolic syndrome [6,7]. AS is characterized by pathological remodeling and calcification processes, leading to varying degrees of morphologic changes of the aortic valve leaflets, including areas of increased thickening, distortion, rigidity, fibrosis, and the presence of bone-enchondral metaplasia [8]. Inflammation causes activation of myofibroblasts, release of cytokines and increased expression of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) [2,9]. Activated myofibroblasts seems to express a variety of defined phenotypes associated with remodeling and repair [10]. *In vitro* studies showed that a subpopulation of myofibroblasts express chondrogenic and osteogenic markers and form calcific nodules in a pro-oxidant medium, while calcium resorption was activated by cells with an osteoclastic phenotype [11].

Recent studies have related gamma-glutamyltransferase (GGT), an enzyme implicated in glutathione metabolism, with the pathogenesis and progression of cardiovascular disease of atherosclerotic nature. Evidence from epidemiological studies suggests that

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plasma GGT is associated with cardiovascular disease risk factors, including diabetes, hypertension, dyslipidemia, and with the metabolic syndrome [12]. GGT is accumulated in atherosclerotic lesions, likely as the result of insudation into the vessel wall of complexes of the enzyme with circulating lipoproteins [13,14], and it has been proposed that GGT may participate in disease progression by modulating one or more of the redox-sensitive processes involved in atherosclerosis [15]. Other findings indicate that GGT may act as a bone resorption factor, stimulating osteoclast formation *in vitro* and promoting osteoporosis in transgenic mice [16,17]. Recent studies have described the occurrence in plasma of four GGT fractions with different molecular weights, whose differential analysis can improve the significance of GGT activity [18,19].

Against this background, we have performed a combined histologic, histochemical and biochemical study of tissue and plasma GGT in a group of patients undergoing aortic valve replacement, in order to evaluate the possible implication of this enzyme activity in aortic valve disease.

2. Materials

2.1. Patients and valve specimens collection

64 consecutive patients undergoing surgical aortic valve replacement for severe and symptomatic calcific AS were enrolled in the study. Exclusion criteria were: any evidence of other coexistent valvular disease, post-rheumatic endocarditis, heart failure, chronic inflammatory or immunologic disease, cancer and osteoporosis. Before surgery, all patients underwent a complete echocardiographic study in order to assess regional and global left ventricular function. To evaluate the degree of stenosis, peak instantaneous transvalvular pressure gradients were determined in all patients. Two-dimensional complete echocardiographic studies were independently performed by two trained echocardiographers, using the Vivid 7 SystemGE with a 4-MHz transducer.

During valve surgery, one of the excised leaflets was immediately frozen in liquid nitrogen for GGT activity determination, the others were fixed in buffered formalin for histological analysis. The research protocol was approved by the local Ethics Committee and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

3. Methods

3.1. Main risk factors and clinical parameters

The cardiovascular risk factors considered in the study were: age, gender, concomitant coronary artery disease, dyslipidemia, diabetes mellitus and hypertension. The following clinical data were evaluated in plasma taken from all patients before surgery: total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides, fibrinogen, brain natriuretic peptide (BNP) and GGT.

3.2. Histology

All samples were fixed in 10% buffered formalin for 24 h and decalcified overnight with formic acid. Valve samples used for histological analysis were taken vertically through the valve cusp near the center of the each leaflets. Sections 5-μm-thick were obtained from paraffin-embedded samples and stained with Hematoxylin–Eosin. Tissue neoangiogenesis and inflammation were valued semiquantitatively according to a scoring system [20]. The presence of lipid deposits or atheroma was also evaluated using semiquantitative analysis (0: absence; 1: small deposits; 2: medium deposits with cholesterol needles; 3: large atheroma-like structure). Tissue calcification was estimated on the basis of an histological morphometric method (direct planimetry by counting point) and expressed as the percentage of calcified tissue respect to the total area of the observed section (mean of 10 sections examined for each case). Bone-enchondral metaplasia was indicated as presence/absence.

3.3. Immunohistochemistry

Immunohistochemical analysis was performed on all samples to detect cells and inflammatory markers: B-lymphocytes (CD20, dilution 1:200; DAKO, Glostrup, Denmark), T-lymphocytes (CD3, dilution 1:1000; Neomarkers, LabVision, Suffolk, UK), monocytes/macrophages (CD68, dilution 1:1000; DAKO), fibroblasts (Vimentin, dilution 1:200; DAKO), smooth muscle cells (smooth β-actin, dilution 1:300; Neomarkers), osteoclasts (TRAP, dilution 1:50; Novocastra Laboratories, UK), GGT (polyclonal prepared in our laboratory [13], dilution 1:900), MMP-2 (dilution 1:50; Chemicon International, Germany), MMP-9 (dilution 1:150; Chemicon International). For detection, the IHC Select Immunoperoxidase Secondary System (Chemicon International, Temecula, CA) was used with a standard peroxidase enzyme substrate (3′,3′-diamonobenzidine, DAB), yielding a dark-brown reaction product. Sections were counterstained with hematoxylin. The expression of GGT, MMP-2 and MMP-9 was graded on a semiquantitative scale, ranging from 0 (no expression) to 3 (most intense expression).

3.4. Fractional GGT activity in plasma and tissue

Fractional GGT was determined in plasma as compared to diseased valve tissue. The amount of tissue available was sufficient to perform biochemical characterization of GGT protein in 12/27 cases. Analysis on plasma-EDTA samples was performed as previously described [19], using a FPLC system (AKTA purifier, GE Healthcare) equipped with a gel-filtration column (Superose 6 HR 10/300 GL, GE Healthcare) and a fluorescence detector (Jasco FP-2020). Fractional GGT area was calculated with the aid of a software (MATLAB Version 7 MathWorks, Inc.) to resolve overlapping peaks. Fraction termed "b-GGT" eluted between 10.0 and 12.0 mL, "m-GGT" between 13.3 and 16.2 mL, "s-GGT" between 17.5 and 20.8 mL, "f-GGT" between 20.8 and 24.0 mL.

For analysis of tissue GGT, frozen valve specimens from the same patients (0.12 ± 0.04 g, range 0.08–0.28 g) were pulverized under liquid nitrogen and homogenized in cold hypotonic lysis buffer (5 mM sodium phosphate buffer pH 7.5, 0.015 M NaCl). Aliquots of crude homogenates were centrifuged at $10{,}000 \times g$ for 20 min at 4 °C and the supernatants were filtered with a 0.45 μm PVDF filter (Millipore). Total GGT activity on crude homogenates and the corresponding soluble fractions were determined using gamma-glutamyl-*p*-nitroanilide as substrate and glycyl-glycine as transpeptidation acceptor [21]. *p*-Nitroaniline formed in the reaction was detected at 405 nm (molar extinction coefficient: 9200 L/(mol cm)). 1 Unit of GGT activity was defined as 1 μmol of substrate transformed/mL/min. Fractional GGT analysis was conducted on the soluble portion of valve homogenates.

3.5. Statistical analysis

Non-parametric Mann–Whitney and Spearman tests were applied to analyse data that were not normally distributed, according to the Kolgomorov–Smirnov test. Unpaired Student's *t* test was used for other comparisons. The significance level was set at $p < 0.05$.

Table 1
Clinical and echocardiographic features of the population studied ($n = 64$).

Age, years	73.0 ± 8.5
Gender, M/F	25/23
Hypertension, *n* (% of total)	45 (70.0)
Diabetes, *n* (% of total)	22 (34.3)
Coronary artery disease, *n* (% of total)	34 (53.0)
Total cholesterol, mg/dL	198.0 ± 51.0
LDL cholesterol, mg/dL	125.0 ± 40.0
HDL cholesterol, mg/dL	50.5 ± 18.0
Triglycerides, mg/dL	107.7 ± 85.1
GGT, U/L	30.9 ± 46.6
CRP, mg/dL	0.7 ± 1.0
Fibrinogen, mg/dL	305.0 ± 84.0
BNP, pg/mL	314.1 ± 301.0
Left ventricular ejection fraction	57.5 ± 11.2
Max peak transvalvular pressure gradient, mm Hg	83.9 ± 29.3
Left ventricular chamber diameter, mm	51.0 ± 8.0
Left ventricular mass index, g/m^2	156.0 ± 39.0

Data are mean ± SD. BNP: brain natriuretic peptide; CRP: C-reactive protein; GGT: gamma-glutamyltransferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

4. Experimental results

4.1. Clinical and echocardiographic findings

The main risk factors, clinical and echocardiographic features of the studied population were summarized in Table 1. Our patients were on average 73 years old and well distributed for gender. They showed hypertension (70.3%), concomitant artery disease (53.0%) and diabetes (34.3%) without significant alterations of serum cholesterol levels. All patients had left ventricular hypertrophy (i.e. LV mass index >135 g/m^2 in man, >110 g/m^2 in women) with increased BNP plasma levels (measured as an index of heart failure). None had left ventricular dilation. Mean ejection fraction was 57.5 ± 11.2.

4.2. Macroscopic findings

All aortic valves examined were tricuspid. Macroscopically, stenotic valves were noticeably thickened, irregular, and showed multiple, contiguous nodules extending from the base toward the middle portion, with preserved commissures.

4.3. Histological findings

By histological analysis we observed that stenotic valves did not preserve structural integrity of the three distinct layers and showed the main features of atherosclerotic lesions: inflammation, neoangiogenesis, lipid deposits and calcifications. The latter were detectable in nodular form, with a "calcium replacement" of valve fibrous tissue. Calcium nodules were located on the aortic side of the leaflet, and were surrounded by thin- and thick-walled neovessels and inflammatory infiltrates. The latter were characterized by macrophages, B- and T-lymphocytes and plasma cells, and were strongly associated with areas of calcification and neoangiogenesis ($p < 0.0001$). Cartilage tissue with chondrocytes and/or ossification areas with bone marrow was observed in 28% (18/64) of stenotic valves. Cholesterol needles, lipid aggregates and foam cells, sometimes arranged in atheroma-like structures, were also found in 69% (44/64) of cases (Fig. 1a). Frequently, lipid deposits appeared as comprised within calcified nodules. Valve lipid deposits were closely associated with decreased serum HDL-c levels ($p = 0.008$), while percentage of tissue calcification (54.82 ± 31.8, mean ± SD) positively correlated with serum BNP ($p = 0.008$) and with bone-enchondral metaplasia ($p = 0.009$).

4.4. Immunohistochemistry

All the remodeling markers studied (MMP-9, MMP-2 and GGT) were expressed in valve leaflets. GGT staining was closely associated with lipid deposits ($p < 0.0001$) and foam cells, localized both in fibrous tissue (Fig. 1b and c) and inside calcified nodules. Dia-

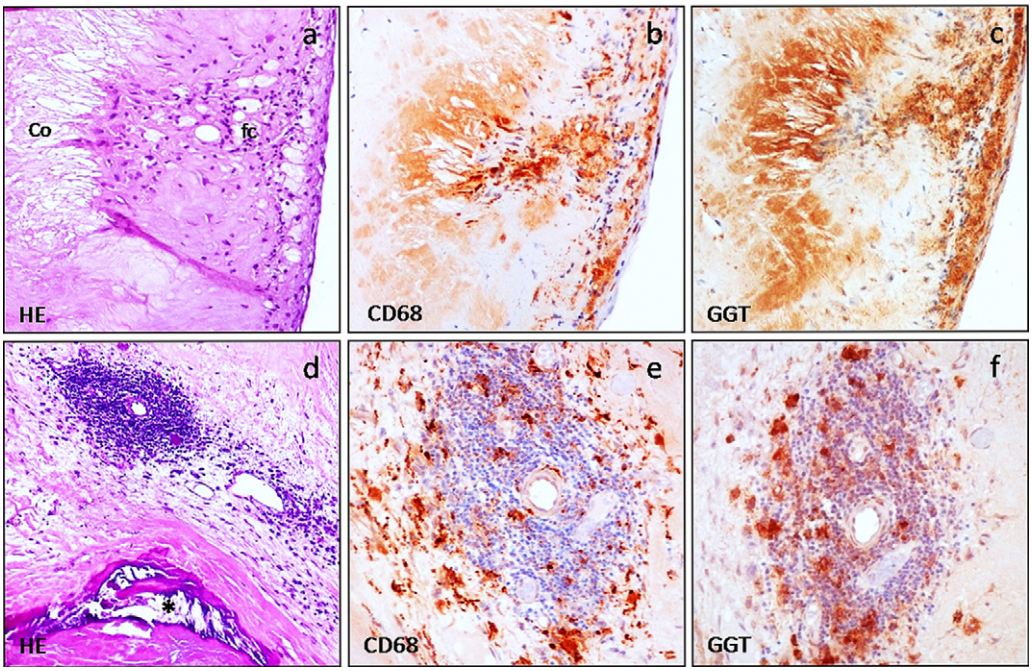


Fig. 1. GGT expression in stenotic aortic valve (I): relationships of GGT with lipids and inflammatory cells. Panel a: cholesterol needles (Co) and foam cells (fc) are arranged in atheroma-like structure. Panels b and c: lipids and foam cells (CD68+) are positive for GGT. Panel d: a large inflammatory infiltrate is located around thin-walled neovessels near a calcified nodule (*). Panels e and f: CD68+ cells within an inflammatory infiltrate express GGT. Hematoxylin–eosin, Immunohistochemistry with DAB revelation, original magnification: 100× (a–d), 200× (e and f).

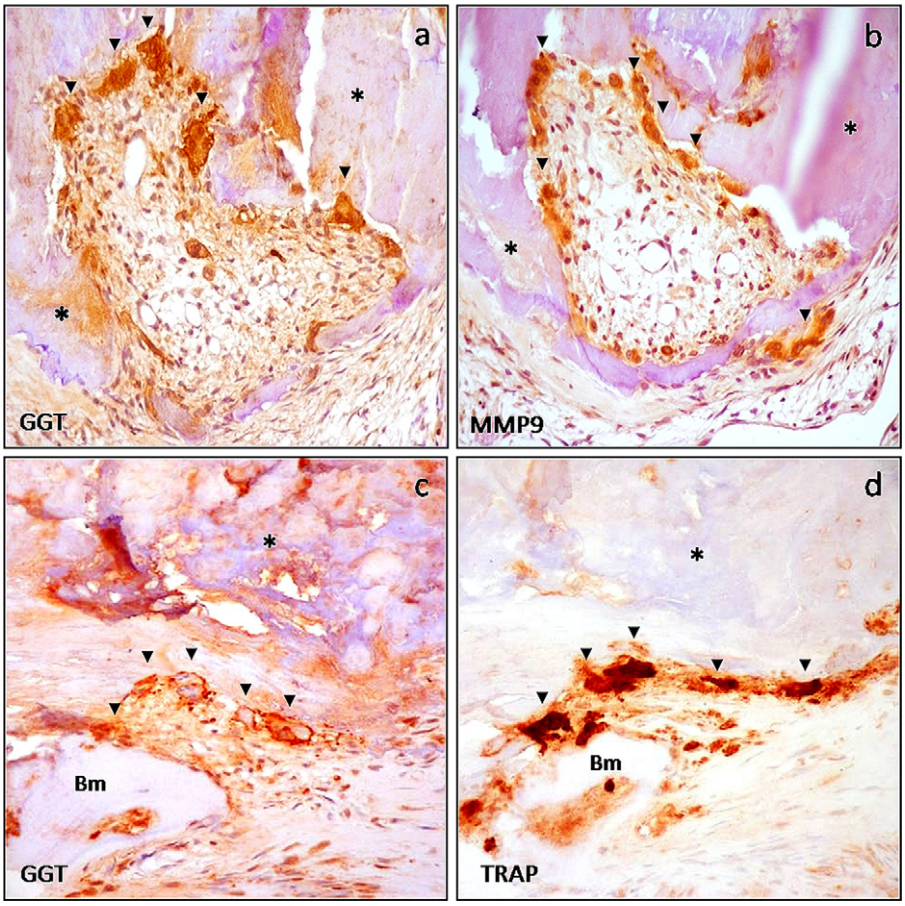


Fig. 2. GGT expression in stenotic aortic valve (II): relationships of GGT with areas of calcification. Panels a and b: multinuclated giant cells (arrowheads), attached on the surface of calcified nodules (*), co-express GGT and MMP-9. Panels c and d: the same GGT-positive cells (arrowheads), observed near bone metaplasia areas (Bm), show an osteoclast phenotype (TRAP+). Immunohistochemistry with DAB revelation, original magnification: 200×.

betes is a major factor in dyslipidemia; however, when analysed in diabetic vs. non-diabetic patients neither serum GGT nor tissue GGT levels were significantly different ($p = 0.67$ and $p = 0.15$, respectively). Tissue GGT was expressed by CD68-positive cells (i.e. monocytes/macrophages) around neovessels, and correlated with neoangiogenesis ($p = 0.005$) (Fig. 1d–f).

A higher magnification analysis of calcified areas revealed the presence of multinucleated giant cells, stained positively for GGT. The same cells also expressed both MMP-9 and Tartrate-Resistant Acid Phosphatase (TRAP), a specific marker for osteoclasts (Fig. 2). Score values of tissue GGT positively correlated with the ones of MMP-9 ($p = 0.001$). MMP-2 was expressed by myofibroblasts in the proximity of mineralization front of calcified nodules (data not shown).

4.5. GGT evaluation in plasma and tissue

Total plasma GGT was not increased, respect to normal values (e.g., men ≤50 U/L, women ≤40 U/L) (Table 1), but higher levels were associated with low HDL-c ($p = 0.028$) and high triglycerides levels ($p = 0.017$) (Fig. 3a and b).

GGT activity of crude valve homogenates was found to range between 9.2 and 199.9 mU/g tissue (mean ± SD: 61.9 ± 46.2 mU/g tissue, $n = 27$). A variable amount of valve GGT was recovered in the soluble portion of the homogenate [22.8 ± 25.1 mU/g tissue (31.5% of total), $n = 27$]. Importantly, higher degrees of valvular stenosis, as judged by peak instantaneous transvalvular pressure gradients, were significantly associated with lower GGT levels both in serum ($p < 0.0003$) and in valve tissue extracts ($p < 0.002$) (Fig. 3c).

Chromatographic fraction analysis of GGT showed that the low molecular weight form (f-GGT) was the most abundant in serum; on the contrary, GGT recovered from valve leaflets was mostly in the form of high molecular weight complexes (b-GGT), ls (Fig. 4). The percentage distribution of GGT forms was significantly different also in the case of s-GGT, higher in plasma. Medium-high molecular weight GGT form (m-GGT) – of which only traces are usually present in plasma of patients within the normal range of total GGT [19] – was undetectable in the valve specimens studied (Fig. 4). Total GGT activity in tissue negatively correlated with the extent of valve calcification ($n = 27$, $p = 0.03$).

5. Discussion

Previous studies have provided the evidence that degenerative aortic valve disease may have an initiating mechanism based on oxidative stress, similar to what is found in atherosclerosis [22,23]. Indeed valve stenosis and atherosclerosis share common risk factors and pathophysiological features. Only half of patients studied however had significant coronary artery disease (53%), while most had hypertension (70.3%). Calcification, the prevalent tissue feature in diseased valves, presented as an active cellular-mediated process strictly associated with inflammation and neoangiogenesis. Bone-enchondral metaplasia with bone marrow was observed in a relevant amount of specimens (28%), in agreement with published studies [24]. Inflammatory infiltration was mainly composed of B- and T-lymphocytes and macrophages. We also observed cholesterol needles, lipid aggregates and foam cells arranged in atheroma-like structures, as previously reported [25,26]. In our

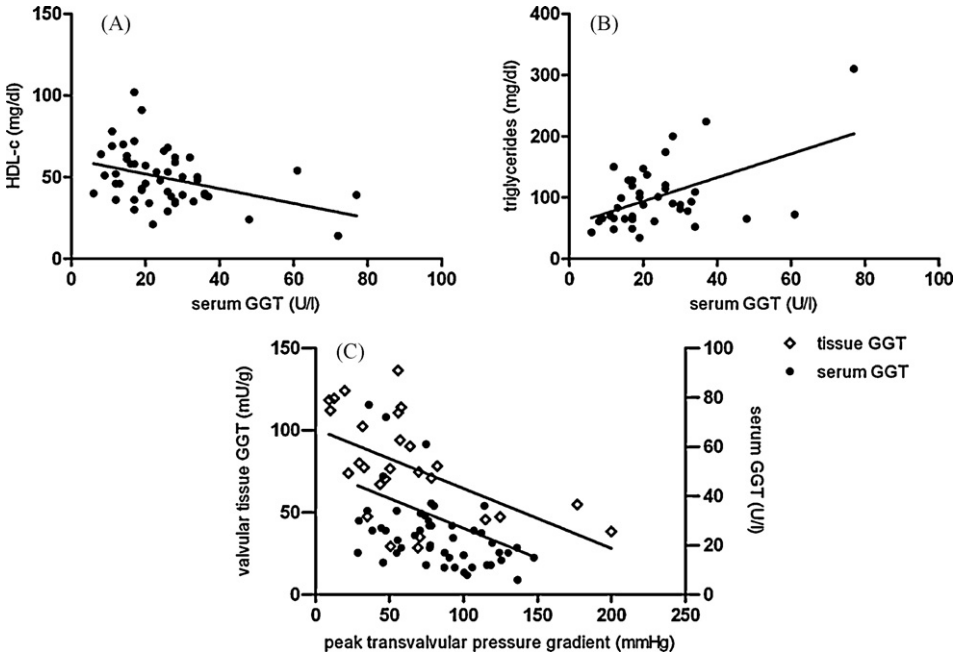


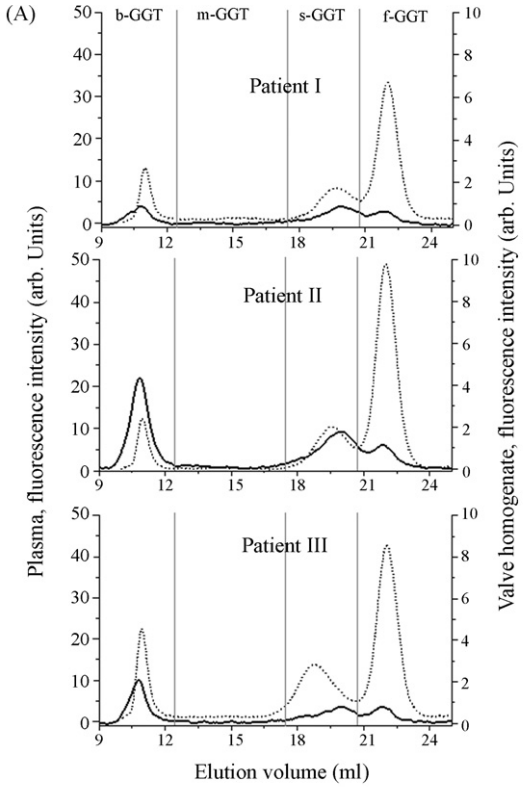
Fig. 3. Correlation of GGT activity levels with plasma lipids profile and severity of aortic valve stenosis. Correlation of total serum GGT with (a) serum HDL-c ($n = 47$; $p = 0.028$) and (b) serum triglycerides ($n = 39$; $p = 0.017$). (c) Negative correlations of GGT levels in serum ($n = 51$; $p < 0.0003$) and valve tissue ($n = 27$; $p < 0.002$) with peak instantaneous transvalvular pressure gradients, determined during echocardiographic evaluation of the patients.

patients total plasma GGT was not increased with respect to normal values (30.9 ± 46.6), but higher levels were associated with an unfavorable lipid profile (low HDL-c and high triglycerides). In a recent study, plasma GGT, in patients within the category of "normal-high" GGT levels (<35 U/L in women, <55 U/L in men), was correlated with mortality from all vascular causes, specifically for ischemic heart disease, while GGT above the reference category was a sensitive predictor of hepatobiliary-related death [27].

As previously documented in studies on atherosclerotic lesions [13], the present study for the first time documents the presence of active GGT enzyme in diseased valve leaflets. The enzyme was associated with lipid deposits and cellular elements, often in proximity of areas of calcification and of neoformed vessels. GGT-positive cells included multinucleated elements also stained for MMP-9, the main metalloproteinase secreted by inflammatory cells [28] and favoring cellular migration and invasion through degradation of matrix components [29,30]. Multinucleated giant cells were attached to calcified nodules and, besides GGT, they also expressed TRAP and CD68, allowing to classify them as osteoclast-like elements. It is conceivable that such multinucleated cells may originate from the differentiation of monocytes/macrophages drawn through neovessels into the tissue, where they could participate in the calcification process and bone metaplasia [31]. Plasma membrane GGT activity plays a critical role in recycling extracellular glutathione and in maintaining intracellular antioxidant homeostasis [32]; therefore, it could preserve the viability and the activity of osteoclast-like cells in a oxidized environment. These observations, enforced by the negative correlation between tissue GGT total activity and calcification, may identify GGT as a resorbing factor in the calcification process of stenotic aortic valve. The role of GGT in tissue calcium balance was previously studied by Niida and co-workers who demonstrated that an overexpression of gamma-glutamyltransferase in transgenic mice accelerated bone resorption and caused osteoporosis [17], probably stimulating the receptor activator of nuclear factor-kappaB ligand (RANKL) [16]. On the other hand, during metabolism of glutathione by GGT the reactive metabolite cysteinyl-glycine is released and its subsequent interactions are responsible for the production of oxygen radicals and generation of a pro-oxidant extracellular environment [33]. Oxidant production is thought to enhance progression of aortic valve calcification [11]. Thus, the fact that GGT accumulation was predominant around calcifying nodules could suggest the participation of GGT-dependent pro-oxidant processes in valvular calcification. *In vitro* studies showed that exogenous superoxide, hydrogen peroxide, or other oxidants increase the number and activity of calcifying vascular cells (CVCs) [34], referred to as a specific subpopulation of cells deriving from (de)differentiation of vascular smooth muscle cells [35], pericytes or mesenchymal cells [36]. In addition, reactive oxygen species (ROS) were shown to mediate the increase in bone morphogenetic protein-2 (BMP2) expression and signaling, thus favoring osteogenesis [37]. On the other hand, ROS derived from NAD(P)H oxidase can also mediate calcium resorption by osteoclasts [38].

GGT activity can be expressed by cellular elements participating in the inflammatory process [39], and cellular infiltrates might be thus the source of GGT accumulating in diseased valves. Nevertheless, as previously suggested [14,18], a significant portion of enzyme found in diseased tissue may as well derive from the insudation of GGT macromolecular forms circulating in serum. The findings of molecular characterization indeed show that three out of four GGT fractions present in serum are also found in valve tissue, although in different proportions. In AS lesions high molecular weight b-GGT form was in fact predominant, while in serum s-GGT and f-GGT were more abundant. Such difference can be explained by the fact that b-GGT is the form released by cells [40], and therefore valve tissue b-GGT may be more abundant due to active secretion by infiltrating macrophages. Actually, b-GGT recovered from AS lesions presented with slightly higher molecular weight, pointing to the possible formation in tissue of even larger protein aggregates as compared to serum. Previous studies in atherosclerotic plaques indicated the tendency of insudated GGT complexes to become less negatively charged and to aggregate, possibly due to protein desialylation effected by inflammatory cells [13].

Concerning the origins of circulating GGT in AS patients, it is doubtful that the diseased valve tissue may contribute to release into the blood significant amounts of the enzyme. The association



(B)

	Total activity (U/L)	b-GGT. %	m-GGT. %	s-GGT. %	f-GGT. %
Plasma GGT	21.3 ± 8.3	12.7 ± 4.4	2.0 ± 1.1	31.1 ± 8.6	54.4 ± 10.7
Valve GGT	5.6 ± 6.9	46.6 ± 37.0 **	N.D.	16.7 ± 17.1 *	7.8 ± 8.8 ***

Fig. 4. GGT fraction analysis in serum and tissue of stenotic aortic valve patients. The four GGT chromatographic fractions, termed b-GGT, m-GGT, s-GGT and f-GGT [19], were determined after homogenization and size exclusion chromatography, in valve tissue (continuous lines, right *y*-axis), and for comparison in sera of the corresponding patients (dotted lines, left *y*-axis). (a) Results for three representative patients are shown (I, II and III). (b) Relative abundance of four GGT molecular isoforms in plasma and valve tissue. N.D. not detectable. Data shown are means ± SD; $^{*}p < 0.02$, $^{**}p < 0.005$, $^{***}p < 0.0001$ (unpaired Student's *t* test).

observed between higher serum GGT values and alterations of the lipid profile (low HDL-c and high triglycerides) might suggest the concomitance of liver dysfunction, and that this may contribute to serum GGT changes. None of the patients however presented with excess alcohol consumption or alterations of other liver parameters. A positive correlation was present between serum GGT and ALT levels ($p < 0.016$, $n = 64$), however serum ALT and AST levels were not associated with either LDL-c, HDL-c or triglycerides.

A limitation of the present study lies in the fact that the observations made in diseased aortic valve tissue could not be compared with normal valves, for obvious reasons. Alternatives could be offered by: specimens obtained during autopsies, in which however post-mortem alterations would make any conclusion unsound or by aortic valves with secondary insufficiency obtained from explanted hearth with dilated ventricule; the latter cannot be considered as healthy tissues. The study was therefore focused on a histological, histochemical and biochemical analysis, as detailed as possible, of AS lesions only.

In conclusion, data reported in the present study suggest the possible implication of GGT activity in the pathogenesis of stenotic aortic valve disease, as previously envisaged for atherosclerosis. Serum GGT levels, usually considered as a marker of alcohol consumption and/or of oxidative stress, have been recently shown to have important prognostic significance in cardiovascular diseases, identifying patients subsets at higher risk of events and requiring specific and enhanced therapeutic efforts [41,42]. Our findings show that higher plasma GGT levels, even if within normal range, are associated with an unfavorable lipid profile (Fig. 3a and b). GGT accumulates in atherosclerotic plaques, where the redox processes deriving from its activity, might participate at several levels in the progression of lesions [15]. The present study, for the first time, documents the accumulation of GGT in lipid deposits of stenotic aortic valves as well, where the same kind of processes can be therefore hypothesized. In serum of healthy subjects, b-GGT is significantly correlated with serum levels of triglycerides [43]. The same was apparent in the AS patients studied (Fig. 3b), in which b-GGT was found to be the form predominantly accumulated in diseased valve tissue.

Altogether, the present study indicates that GGT activity may play a complex role in the pathogenesis of aortic valve stenosis. In fact, the negative correlation of serum and tissue GGT levels with severity of valve stenosis (peak transvalvular pressure gradients), the negative association of tissue GGT levels with valvular calcification, as well as the localization of GGT expression in osteoclast-like elements in the proximity of calcified nodules, suggest that the enzyme might play as a resorbing factor in calcification remodeling, in apparent agreement with the recently envisaged implication of GGT in bone homeostasis [16,17]. Further studies are thus warranted in order to elucidate (i) the connections linking plasma lipid patterns to alterations of plasmatic GGT forms, (ii) the molecular mechanism of GGT involvement in valve calcification remodeling, and (iii) the significance of these aspects for the prognosis of aortic valve disease.

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Conflict of interest

None declared.

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