Galectin-3 Predicts Long-Term Cardiovascular Death in High-Risk Patients With Coronary Artery Disease

Giuseppe Maiolino,* Giacomo Rossitto,* Luigi Pedon, Maurizio Cesari, Anna Chiara Frigo, Matteo Azzolini, Mario Plebani, Gian Paolo Rossi

Objective—Galectin-3 (Gal-3) can affect atherogenesis by multiple mechanisms, but it remains scarcely known whether plasma Gal-3 levels predict cardiovascular events in patients with coronary artery disease. Therefore, we investigated whether Gal-3 predicts cardiovascular death in patients with coronary artery disease of the Genetic and ENvironmental factors In Coronary Artery disease study.

Approach and Results—In a prospective cohort study, we measured the plasma levels of Gal-3 in 1013 randomly selected patients who underwent coronary angiography and long-term follow-up to assess incident cardiovascular events. The primary end points were (1) cardiovascular death and (2) a composite of cardiovascular death, acute coronary syndrome, and stroke. Secondary end points entailed (1) acute myocardial infarction, (2) stroke, and (3) a composite fatal ischemic event including fatal myocardial infarction and stroke. The effect of Gal-3 on prognosis was assessed using Kaplan–Meier analysis and multivariate Cox’s regression. During long-term follow-up (median, 7.2 years), 115 cardiovascular deaths occurred (15.2%), more commonly in the high Gal-3 tertile (25.2%) than in the intermediate and the low tertiles (13.6% versus 7.5%, respectively; \( P<0.001 \)). The adverse prognostic effect of high Gal-3 was confirmed in subgroup analysis of the patients with angiographically documented coronary artery disease and also of those with a normal left ventricular ejection fraction. At multivariate analysis, Gal-3 was a predictor of cardiovascular mortality (hazard ratio, 1.79; 95% confidence interval, 1.10–2.93; \( P<0.020 \)) along with age, left ventricular ejection fraction, and coronary atherosclerotic burden.

Conclusions—in high cardiovascular risk patients referred for coronary angiography Gal-3 is a strong independent predictor of cardiovascular death. (Arterioscler Thromb Vasc Biol. 2015;35:00-00. DOI: 10.1161/ATVBAHA.114.304964.)

Key Words: atherosclerosis ■ coronary artery disease ■ galectin-3 ■ prognosis ■ prospective studies

Galectin-3 (Gal-3) belongs to a family of soluble β-galactoside binding lectins1 that reside in the nucleus and cytoplasm of several cell types as well as in the extracellular space.2–4 It has multiple actions that can be relevant for promoting vascular damage5–7 and cardiovascular fibrosis.8,9 Accordingly, in patients with heart failure plasma Gal-3 level has been shown to be the best short-term predictor of events,10,11 thus leading to incorporation of this measurement in the current American Heart Association heart failure guidelines for risk stratification purposes of such patients.12

Some actions of Gal-3, as monocytes chemoattraction, enhancement of phagocytosis,5,6 and induction of vascular smooth cells proliferation,7 can play an important role in atherogenesis. However, in spite of these multiple proatherogenic actions, the role of Gal-3 in atherosclerosis has been scarcely investigated in clinical studies thus far.13–17 Therefore, in the prospective branch of the Genetic and ENvironmental factors in Coronary Artery disease (GENICA) study, which enrolled high cardiovascular risk patients referred for coronary angiography for suspected coronary artery disease (CAD), we sought to challenge the hypothesis that plasma Gal-3 levels predict cardiovascular deaths and events during long-term follow-up by giving due consideration to many other cardiovascular risk factors and potential confounders.

Methods

Materials and Methods are available in the online-only Data Supplement. For the study flow diagram, please refer to Figure 1.

Results

Clinical Characteristics

Ninety-six percent of the enrolled patients were at high cardiovascular risk by the ATP III NCEP criteria,18 which means

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they had ≥20% risk of major cardiovascular events at 10 years. Table I and Table I in the online-only Data Supplement show their features divided into Gal-3 tertiles. Age, sex, serum glucose, sodium, potassium and creatinine levels, estimated glomerular filtration rate, left ventricular ejection fraction (LVEF), homocysteine, low-density lipoprotein cholesterol, medical history, and use of medications at admission differed significantly across the Gal-3 tertiles.

Results were overall similar when the patients with angiographically demonstrated CAD (n=782; Tables II and III in the online-only Data Supplement) and the patients with CAD and normal LVEF (n=595; Table 2 and Table IV in the online-only Data Supplement) were examined. In these subgroups the only additional differences entailed CAD Duke Index Score and high-density lipoprotein cholesterol in the CAD group and high-density lipoprotein cholesterol in the normal LVEF group.

Regression analysis showed that plasma Gal-3 levels were directly related to sex (β=0.083), body mass index (β=0.134), diuretic therapy (β=0.071), triglycerides (β=0.061), and plasma homocysteine (β=0.109), and inversely related to estimated glomerular filtration rate (β=−0.292), and LVEF (β=−0.128). These variables explained 18% of plasma Gal-3 variance (adjusted R²=0.183; F=32.6; P<0.0001; Table 3). Gal-3 was significantly associated with the atherosclerotic burden only at univariate analysis (p=0.104; P=0.004).

### Cardiovascular Events at Follow-Up in the Whole Cohort

Follow-up (median, 7.2 years; range, 1.0–12.7 years) data were available in 75% of the 1013 patients. At multiple regression analysis the cases lost did not differ significantly from those available at follow-up (Table V in the online-only Data Supplement). Among the latter patients, 115 (15.2%) had a cardiovascular death, 212 (28.0%) a major cardiovascular event, and 57 (7.5%) a fatal ischemic event. The major cardiovascular events by Gal-3 tertiles are shown in Figure 2.

At univariate analysis of the whole cohort, patients in the highest Gal-3 tertile had more cardiovascular deaths (25.2%) than those in the intermediate and in the lower tertiles (13.6% and 7.5%, respectively; P<0.001). They also had higher cardiovascular events (35.0% versus 29.6% versus 21.0%, respectively; P<0.001), and fatal ischemic events (11.8% versus 6.7% versus 4.1%, respectively; P=0.001; Figure 3 and Figures I and II in the online-only Data Supplement).

Cox’s multivariate analyses were undertaken because of the imbalanced distribution of risk factors and relevant covariates across Gal-3 tertiles. After these adjustments Gal-3 remained an independent predictor of cardiovascular death (hazard ratio [HR], 1.79; 95% confidence interval [CI], 1.10–2.93; P=0.020), along with age (HR, 1.47; 95% CI, 1.19–1.81; P<0.0001), LVEF (HR, 0.68; 95% CI, 0.57–0.82; P<0.0001), and CAD Duke Index Score (HR, 1.14; 95% CI, 1.03–1.26; P=0.012; Table 4).

Gal-3 was not a significant predictor of cardiovascular events according to the P value for significance prespecified for multiple testing (Table VI in the online-only Data Supplement). However, it was borderline significant for fatal ischemic events (HR, 2.28; 95% CI, 1.09–4.74; P=0.028), along with age, serum sodium, angiotensin-converting enzyme inhibitor treatment, heparin, and digoxin therapy (Table VII in the online-only Data Supplement).

The Gal-3 cutoff value for optimizing prediction was 16.6 ng/mL (AUC, 0.689; 95% CI, 0.654–0.722; P<0.0001) for cardiovascular death, and 13.9 ng/mL (AUC, 0.595; 95% CI, 0.559–0.630; P<0.0001) for cardiovascular events (Figure III in the online-only Data Supplement).
events prediction in patients with CAD were 20.3 ng/mL (AUC, 0.703; 95% CI, 0.665–0.739; P<0.0001) and 13.9 ng/mL (AUC, 0.578; 95% CI, 0.537–0.617; P<0.004), respectively.

Finally, to address the possibility that our results could be driven by the known adverse prognostic effect of Gal-3 in patients with heart failure, we analyzed only the cohort of subjects with angiographically proven CAD and preserved LVEF (>50%; n=595). Follow-up data were available for 465 patients (78%) and in this group 40 (8.6%) had a cardiovascular death, 106 (22.8%) a major cardiovascular event, and 27 (5.8%) a fatal ischemic event (Figure VI in the online-only Data Supplement). Consistently with the previous findings the highest Gal-3 tertile patients had more cardiovascular deaths (13.4% versus 8.8% versus 4.3%, respectively; P=0.015) also in this subgroup. Moreover, they showed a nonsignificant trend toward more fatal ischemic events (9.2% versus 5.1% versus 3.0%, respectively; P=0.05) compared with the lower Gal-3 tertiles. Cardiovascular events did not differ significantly (24.1% versus 26.2% versus 19.1%, respectively; P=0.2; Figure 4).

Cox regression analysis confirmed that Gal-3 was an independent predictor of cardiovascular death (HR, 3.78; 95% CI, 1.51–9.51; P=0.005) along with CAD Duke Index Score, history of percutaneous transluminal coronary angioplasty, estimated glomerular filtration rate, and digoxin therapy (Table 5). Moreover, Gal-3 independently predicted fatal ischemic events (HR, 5.44; 95% CI, 1.86–15.93; P=0.002) (Table IX in the online-only Data Supplement), albeit not cardiovascular events. Finally, at ROC curves analysis the Gal-3 cutoff value for cardiovascular death prediction was 27.7 ng/mL (AUC, 0.625; 95% CI, 0.579–0.669; P<0.01).

Discussion
This prospective cohort study with a long and comprehensive follow-up showed that plasma Gal-3 level is an independent predictor of cardiovascular death in patients with CAD.
predictor of cardiovascular mortality in patients with CAD without overt LV dysfunction. This finding extends those obtained in patients with heart failure.10,11

Association of Gal-3 With Coronary Atherosclerosis

Studies on small cohorts comprising variable proportions of patients with CAD previously reported an association of Gal-3 with prevalence of CAD19 and a borderline significant association with the number of affected vessels.13,20 At variance, we found only a weak correlation not confirmed at multivariate analysis, between Gal-3 levels and the angiographically ascertained coronary atherosclerotic burden in this large cohort of patients with CAD.

Considering the blunted development of atherosclerosis found in apolipoprotein E/Gal-3 double knockout mice, as compared with apolipoprotein E knockout mice,21 the multiple proatherogenic actions of Gal-3,5–7 and the detection of Gal-3 human atherosclerotic plaques,22 this was an unexpected finding that re-emphasizes the divergence between predictors of CAD and of cardiovascular events (see below).

Prognostic Effect of Gal-3

A major novel accomplishment of this study was the finding that in a cohort of patients at high cardiovascular risk,18 selected for

Table 2. Demographic and Clinical Characteristics of the CAD Subjects With Preserved LVEF Classified by Galectin-3 Tertiles

<table>
<thead>
<tr>
<th>Variable</th>
<th>Primary (n=199; 33.4%)</th>
<th>Secondary (n=201; 33.8%)</th>
<th>Tertiary (n=195; 32.8%)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>60 (53–66)*</td>
<td>65 (57–71)†</td>
<td>69 (62–74‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, men (%)</td>
<td>178 (89.4)</td>
<td>162 (80.6)</td>
<td>137 (70.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers/smokers/ex, %</td>
<td>66/38/93 (34/19/47)</td>
<td>78/26/97 (39/13/48)</td>
<td>79/23/93 (41/12/48)</td>
<td>0.215</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>110 (55.3)</td>
<td>121 (60.2)</td>
<td>127 (65.1)</td>
<td>0.095</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>134±18</td>
<td>135±18</td>
<td>137±18</td>
<td>0.253</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>79±10</td>
<td>78±9</td>
<td>78±10</td>
<td>0.723</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>19 (9.6)</td>
<td>34 (17.0)</td>
<td>42 (21.6)</td>
<td>0.064</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.9±3.2</td>
<td>26.4±3.0†</td>
<td>27.3±4.4‡</td>
<td>0.050</td>
</tr>
<tr>
<td>Heart disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricular EF, %</td>
<td>67 (60–73)</td>
<td>67 (62–73)†</td>
<td>65 (59–72)</td>
<td>0.018</td>
</tr>
<tr>
<td>Atherosclerotic burden (Duke score)</td>
<td>32 (23–42)</td>
<td>37 (23–48)</td>
<td>37 (23–48)</td>
<td>0.333</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>88 (71–97)‡</td>
<td>88 (71–97)†</td>
<td>88 (80–106‡</td>
<td>0.007</td>
</tr>
<tr>
<td>eGFR, mL/min</td>
<td>90.2±26.9*</td>
<td>79.9±24.2‡</td>
<td>74.1±29.3‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum K+, mmol/L</td>
<td>4.2±0.4</td>
<td>4.2±0.4</td>
<td>4.3±0.4</td>
<td>0.062</td>
</tr>
<tr>
<td>Serum Na+, mmol/L</td>
<td>140±2</td>
<td>140±2†</td>
<td>139±2‡</td>
<td>0.002</td>
</tr>
<tr>
<td>Serum glucose, mmol/L</td>
<td>6.2±1.8</td>
<td>6.3±2.0</td>
<td>6.5±2.1</td>
<td>0.339</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>203 (181–237)</td>
<td>207 (181–237)</td>
<td>201 (175–220)</td>
<td>0.087</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>46 (40–51)</td>
<td>46 (40–53)†</td>
<td>43 (37–48‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>131 (115–157)</td>
<td>131 (111–156)</td>
<td>131 (111–147)</td>
<td>0.130</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>125 (91–173)</td>
<td>121 (88–162)</td>
<td>127 (95–177)</td>
<td>0.474</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>11.6±7.4</td>
<td>11.6±6.8†</td>
<td>14.0±8.3‡</td>
<td>0.003</td>
</tr>
<tr>
<td>Study data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galectin-3, ng/mL</td>
<td>10.7 (9.6–11.7)*</td>
<td>14.8 (13.7–15.7)†</td>
<td>20.4 (18.1–24.1)‡</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD, absolute numbers (percentage), or median and interquartile range; comparisons across Galectin-3 tertiles were made by ANOVA and Bonferroni tests, after log or square root transformation if needed, or χ², as appropriate. BP indicates blood pressure; BMI, body mass index; CAD, coronary artery disease; EF, ejection fraction; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; K+, potassium; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; and Na⁺, sodium. Bonferroni test: *I vs II tertile, †II vs III tertile, and ‡I vs III tertile. P<0.05 for significance.

Table 3. Stepwise Linear Regression Analysis of Determinants of Plasma Galectin-3

<table>
<thead>
<tr>
<th>Variables in the model</th>
<th>β</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galectin-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.083</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI</td>
<td>0.134</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment with diuretics</td>
<td>0.071</td>
<td>0.011</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.061</td>
<td>0.021</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>0.109</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glomerular filtration rate</td>
<td>−0.292</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left ventricular EF</td>
<td>−0.128</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Significant predictors of galactin-3 were sex, BMI, diuretics, triglycerides, plasma homocysteine, estimated glomerular filtration rate, and left ventricular EF. Analysis performed in the whole cohort, n=1013. BMI indicates body mass index; and EF, ejection fraction.
suspected CAD, high Gal-3 levels predicted cardiovascular mortality (Figure 3) independently of major risk factors. This contention is strengthened by the improved prediction of cardiovascular death by adding Gal-3 to well-established risk factors, like the coronary atherosclerotic burden, at Cox’s regression analysis (Table 4).

The prognostic role of Gal-3 was well documented previously in patients with heart failure,9 where Gal-3 was identified as the best short-term predictor of cardiovascular death by adding Gal-3 to well-established risk factors, like the coronary atherosclerotic burden, at Cox’s regression analysis (Table 4).

Figure 2. Cardiovascular events. Cardiovascular (CV) death (top), CV events (middle), and fatal ischemic events (bottom) rate by tertiles of Gal-3 in the whole cohort (the number of events is shown above each column). CV deaths \( \chi^2=31.9, P<0.001 \); CV events \( \chi^2=15.0, P=0.002 \); and fatal ischemic events \( \chi^2=10.9, P=0.004 \).

Figure 3. Cardiovascular (CV) death–free (top), CV event–free (middle), and fatal ischemic event–free survival (bottom) in the whole cohort. Kaplan–Meier curves show that patients in the high Gal-3 tertile (dashed line) had a significantly higher CV deaths, CV events, and fatal ischemic events than the patients in the mid (dotted line) and low (solid line) Gal-3 tertiles.

The strong prognostic role of Gal-3 in spite of its weak association with CAD atherosclerotic burden deserves some comments: fatal myocardial infarction and atherothrombotic stroke are known to be triggered by rupture of vulnerable atherosclerotic plaques, which is enhanced by factors activating matrix metalloproteinases. The determinants of plaque destabilization, for example, of cardiovascular events, and of plaque growth, for example, of the atherosclerotic burden, are known to differ although they can overlap. Our present results suggest that Gal-3 can contribute more substantively to plaque destabilization than to plaque growth. Of interest, similar discrepancies between some prognostic determinants and the CAD atherosclerotic burden were previously noted both in the GENICA26–28 and in other studies.29,30
Subgroup Analyses

Given the prognostic impact of Gal-3 in patients with heart failure,10,23,31 many of which have CAD as underlying cause, it might be argued that the adverse effect of Gal-3 on cardiovascular death–free survival in our cohort was because of inclusion of patients with heart failure. This contention was, however, not supported by our findings in a subgroup analysis of the patients with preserved LVEF, where high Gal-3 levels were also associated with higher cardiovascular deaths (Table 5).

A sensitivity analysis of the patients with angiographically documented CAD showed a practically identical adverse prognostic effect of Gal-3. Hence, with the strength of a prospective cohort study design and of a large cohort of consecutively enrolled patients, our data demonstrate that Gal-3 predicts cardiovascular mortality in patients with CAD regardless of the presence or absence of left ventricular systolic dysfunction. This finding is at variance with previous reports in the general population,24 in smaller CAD cohorts,13–16 and in a large cohort of patients with angiographically demonstrated CAD.32

A prognostic role of Gal-3 on nonfatal cardiovascular events could not, however, be confirmed at variance with previous studies,13–16 possibly because in those cohorts most of the effect on cumulative end points was driven by heart failure–related events. Another possibility is that adjustment for the Duke CAD Index Score, one of the strongest predictors of cardiovascular events and death in patients with CAD, might have concealed an effect of Gal-3 on these end points in our study. The use of this index was never exploited previously in studies of Gal-3.13,16

<table>
<thead>
<tr>
<th>Table 4. Predictors of Cardiovascular Mortality in the Whole Cohort at Cox Regression Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular Mortality: Whole Cohort</td>
</tr>
<tr>
<td>HR</td>
</tr>
<tr>
<td>Model 1</td>
</tr>
<tr>
<td>Age (√ increase)</td>
</tr>
<tr>
<td>LVEF (√ increase)</td>
</tr>
<tr>
<td>CAD Duke Index Score</td>
</tr>
<tr>
<td>Digoxin therapy</td>
</tr>
<tr>
<td>Galectin-3 (Ln increase)</td>
</tr>
<tr>
<td>Model 2</td>
</tr>
<tr>
<td>Age (√ increase)</td>
</tr>
<tr>
<td>LVEF (√ increase)</td>
</tr>
<tr>
<td>LDLc (Ln increase)</td>
</tr>
<tr>
<td>Galectin-3 (1 Ln increase)</td>
</tr>
</tbody>
</table>

Model 1, adjusted for age; sex; left ventricular ejection fraction; coronary atherosclerotic burden (Duke Score); high-density lipoprotein cholesterol and LDL-C; body mass index; hypertension; diabetes mellitus; serum sodium; serum potassium; estimated glomerular filtration rate; homocysteine; history of myocardial infarction, previous revascularization by percutaneous transluminal coronary angioplasty, peripheral vascular disease; and use of angiotensin-converting enzyme inhibitors, β-blockers, diuretics, digoxin, and heparin. Model 2, adjusted for aforementioned variables excluding therapy. P for significance <0.05.

Cl indicates confidence interval; CAD, coronary artery disease; HR, hazard ratio; LDL-C, calculated low-density lipoprotein cholesterol; and LVEF, left ventricular ejection fraction.

Subgroup Analyses

Gal-3 Levels Predictors

Our study allowed identification of sex, body mass index, triglycerides, and estimated glomerular filtration rate as predictors of Gal-3 (Table 3), which concurs with data in the general population.24 Additional identified predictors, as diuretic treatment and low LVEF, likely reflect the higher levels of Gal-3 in patients with heart failure. Of note, plasma homocysteine was also found to be associated with plasma Gal-3 (Table 3). This could be mediated by either a low glomerular filtration rate or a low LVEF, both of which are related with plasma homocysteine.33,34 It is worth mentioning, however, that homocysteine can increase Gal-3 through its effect on nuclear factor-kB, a known inducer of Gal-3.35,36 This finding could suggest a beneficial effect of homocysteine-lowering treatment, as folates supplementation, in patients with high Gal-3 levels.
Table 5. Predictors of Cardiovascular Mortality at Cox Regression Analysis in Patients With CAD and Preserved Left Ventricular Ejection Fraction

<table>
<thead>
<tr>
<th>Cardiovascular Mortality: Patients With CAD and Preserved LVEF</th>
<th>HR</th>
<th>95% CI</th>
<th>Wald</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1&lt;br&gt; CAD Duke Index Score</td>
<td>1.14</td>
<td>(1.03–1.26)</td>
<td>4.26</td>
<td>0.039</td>
</tr>
<tr>
<td>eGFR (Ln increase)</td>
<td>0.36</td>
<td>(0.13–0.99)</td>
<td>3.95</td>
<td>0.047</td>
</tr>
<tr>
<td>History of PTCA</td>
<td>4.95</td>
<td>(1.86–13.16)</td>
<td>10.22</td>
<td>0.001</td>
</tr>
<tr>
<td>Digoxin therapy</td>
<td>8.13</td>
<td>(2.56–25.64)</td>
<td>12.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Galectin-3 (Ln increase)</td>
<td>3.78</td>
<td>(1.50–9.51)</td>
<td>8.00</td>
<td>0.005</td>
</tr>
<tr>
<td>Model 2&lt;br&gt; History of PTCA</td>
<td>4.03</td>
<td>(1.63–9.90)</td>
<td>9.18</td>
<td>0.002</td>
</tr>
<tr>
<td>Galectin-3 (Ln increase)</td>
<td>3.54</td>
<td>(1.38–9.12)</td>
<td>6.88</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Limitations and Strengths of the Study
Our results pertain to a high-risk population of white patients referred for coronary artery angiography. Therefore, they need to be verified in patient cohorts with different risk profiles and ethnicity. It might also be argued that the loss of 25% of the patients at follow-up could have biased our results. However, a selection bias is unlikely as these cases lost did not differ from those available at follow-up for any baseline features.

Conclusions
In this prospective cohort study in patients with angiographically demonstrated CAD at high cardiovascular risk Gal-3 was a major predictor of cardiovascular mortality. The robustness of this conclusion was confirmed in patients with preserved LVEF and also after adjustments for several covariates that are known to affect prognosis in patients with CAD. Clinical trials designed to test the effects of Gal-3 inhibitors as modified citrus pectin are therefore warranted to prove causality between Gal-3 and progression/complication of coronary atherosclerosis.


**Significance**

This is the first study to show that plasma galectin-3 is a predictor of cardiovascular death independent of the other major risk factors in high-risk patients referred for coronary artery angiography. More specifically, in the patients with coronary artery disease, plasma galectin-3 levels >20.3 ng/mL were associated with an increased risk of cardiovascular death. The prognostic usefulness of galectin-3 was not simply because of heart failure as it was confirmed by a sensitivity analysis in the subgroup of patients with normal left ventricular ejection fraction.
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GALECTIN-3 PREDICTS LONG TERM CARDIOVASCULAR DEATH
IN HIGH-RISK CORONARY ARTERY DISEASE PATIENTS

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Giuseppe Maiolino*², MD, PhD, Giacomo Rossitto*², MD, Luigi Pedon¹, MD, Maurizio Cesari², MD, PhD, Anna Chiara Frigo³, MS, Matteo Azzolini², MD, Mario Plebani⁴, MD, Gian Paolo Rossi², MD.

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SUPPLEMENTAL MATERIAL
Figures 6, Tables 9

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E-mail: gianpaolo.rossi@unipd.it
Fig. I suppl: Occurrence of Acute Myocardial Infarction (AMI) in the whole cohort (top), CAD patients (mid) and CAD patients with preserved left ventricular ejection fraction (bottom). Kaplan-Meier curves for AMI in the high (dashed line), mid (dotted line) and low (solid line) Gal-3 tertiles.
Fig. II suppl: Occurrence of Stroke in the whole cohort (top), CAD patients (mid) and CAD patients with preserved left ventricular ejection fraction (bottom). Kaplan-Meier curves for Stroke in the high (dashed line), mid (dotted line) and low (solid line) Gal-3 tertiles.
Fig. III supplemental: Receiver-operating characteristic (ROC) curve for Galectin-3. The ROC analysis for Gal-3 showed a c-statistic of 0.689 (p < 0.0001) (solid line) with a 95% confidence interval of 0.654-0.722 (dotted lines). Y.I.: Youden Index.
Fig. IV suppl: Cardiovascular events, CAD patients. Cardiovascular death and events rate in patients with angiographically documented CAD by tertiles of Gal-3 (the absolute number of events is shown above each column). CV deaths $X^2 = 26.3$, $p < 0.001$; CV events $X^2 = 6.1$, $p = 0.047$; Fatal ischemic events $X^2 = 7.8$, $p = 0.02$. CV: cardiovascular.
Fig. V suppl: Cardiovascular deaths (top), CV events (mid), and fatal ischemic events (bottom) in the CAD patients. Kaplan-Meier curves show that patients in the high Gal-3 tertile (dashed line) had a significantly higher cardiovascular (CV) deaths, CV events, and fatal ischemic events than the patients in the mid (dotted line) and low (solid line) Gal-3 tertiles.
Fig. VI suppl: Cardiovascular events, CAD patients with preserved LVEF.
Cardiovascular death and events rate in patients with angiographically proven CAD and preserved LVEF (> 50%) by tertiles of Gal-3 (the absolute number of events is shown above each column). CV deaths $X^2 = 4.2$, $p = 0.118$; CV events $X^2 = 1.5$, $p = 0.473$; Fatal ischemic events $X^2 = 5.0$, $p = 0.081$. CV: cardiovascular.
### Supplemental Table I. Past medical history and medications at baseline of the whole cohort classified by Galectin-3 tertiles.

Results are expressed as absolute number (percentage); comparisons across Galectin-3 tertiles were made by $X^2$. AMI, acute myocardial infarction; Bypass, coronary artery bypass; Hx, history; PTCA, percutaneous transluminal coronary angioplasty; ACE, angiotensin converting enzyme. $n = 1013$.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$1^\circ$ (n=348, 34.4%)</th>
<th>$2^\circ$ (n=329, 32.5%)</th>
<th>$3^\circ$ (n=336, 33.2%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical condition/Hx (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hx of stroke</td>
<td>4 (1.2)</td>
<td>5 (1.5)</td>
<td>9 (2.7)</td>
<td>0.293</td>
</tr>
<tr>
<td>Hx of AMI</td>
<td>105 (30.2)</td>
<td>120 (36.6)</td>
<td>124 (36.9)</td>
<td>0.112</td>
</tr>
<tr>
<td>Hx of Bypass</td>
<td>35 (10.1)</td>
<td>32 (9.7)</td>
<td>29 (8.7)</td>
<td>0.811</td>
</tr>
<tr>
<td>Hx of PTCA</td>
<td>34 (9.8)</td>
<td>23 (7.1)</td>
<td>14 (4.2)</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>Medications at baseline (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>110 (32.3)</td>
<td>95 (29.1)</td>
<td>105 (31.8)</td>
<td>0.645</td>
</tr>
<tr>
<td>Oral antiplatelet agents</td>
<td>252 (73.9)</td>
<td>237 (72.7)</td>
<td>220 (66.7)</td>
<td>0.088</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>136 (39.9)</td>
<td>128 (39.3)</td>
<td>113 (34.2)</td>
<td>0.259</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>142 (41.6)</td>
<td>122 (37.4)</td>
<td>116 (35.2)</td>
<td>0.213</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>138 (40.5)</td>
<td>140 (42.9)</td>
<td>186 (56.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heparin</td>
<td>67 (19.6)</td>
<td>84 (25.8)</td>
<td>60 (18.2)</td>
<td>0.041</td>
</tr>
<tr>
<td>Diuretics</td>
<td>69 (20.2)</td>
<td>97 (29.8)</td>
<td>161 (48.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Digoxin</td>
<td>25 (7.3)</td>
<td>35 (10.7)</td>
<td>60 (18.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
### Supplemental Table II. Demographic and clinical characteristics of the CAD subjects classified by Galectin-3 tertiles (n=782).

Results are expressed as mean ± SD, absolute numbers (percentage), or median and interquartile range; comparisons across Galectin-3 tertiles were made by ANOVA and Bonferroni tests, after log or square root transformation if needed, or X², as appropriate. BMI, body mass index; eGFR, estimated glomerular filtration rate; K⁺, potassium; Na⁺, sodium; BP, Blood Pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; EF, ejection fraction. Bonferroni test: * I vs II tertile, # II vs III tertile, § I vs III tertile. P < 0.05 for significance. *Systolic and diastolic BP and BMI values did not differ across Gal-3 tertiles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1° (n=267, 34.2%)</th>
<th>2° (n=255, 32.6%)</th>
<th>3° (n=260, 33.2%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>61 [54-67]</td>
<td>65 [57-71]</td>
<td>69 [63-74]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender M (%)</td>
<td>240 (89.9)</td>
<td>203 (79.6)</td>
<td>193 (74.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Smokers/Smokers/Ex (%)</td>
<td>85/51/130 (32/19/49)</td>
<td>96/36/123 (38/14/48)</td>
<td>97/35/128 (37/14/49)</td>
<td>0.306</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>142 (53.0)</td>
<td>156 (61.2)</td>
<td>171 (65.8)</td>
<td>0.018</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>133 ± 18</td>
<td>135 ± 18</td>
<td>134 ± 18</td>
<td>0.214</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>78 ± 10</td>
<td>77 ± 9</td>
<td>78 ± 10</td>
<td>0.237</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>36 (13.5)</td>
<td>37 (14.7)</td>
<td>54 (20.9)</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>Heart disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Ventricular EF (%)</td>
<td>65 [56-71]</td>
<td>64 [54-72]</td>
<td>59 [47-68]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum Creatinine (µmol/L)</td>
<td>88 [71-97]</td>
<td>88 [71-97]</td>
<td>96 [80-115]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>87.5 ± 26.7</td>
<td>79.9 ± 24.9</td>
<td>70.9 ± 30.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum K⁺ (mmol/L)</td>
<td>4.2 ± 0.4</td>
<td>4.2 ± 0.4</td>
<td>4.3 ± 0.5</td>
<td>&lt;0.012</td>
</tr>
<tr>
<td>Serum Na⁺ (mmol/L)</td>
<td>140 ± 2</td>
<td>140 ± 2</td>
<td>139 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum Glucose (mmol/L)</td>
<td>6.2 ± 2.1</td>
<td>6.2 ± 1.9</td>
<td>6.6 ± 2.3</td>
<td>0.084</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>202 [181-237]</td>
<td>206 [177-234]</td>
<td>199 [173-226]</td>
<td>0.035</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
<td>46 [40-51]</td>
<td>46 [40-52]</td>
<td>43 [36-49]</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dL)</td>
<td>131 [114-157]</td>
<td>131 [111-154]</td>
<td>131 [109-144]</td>
<td>0.014</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>126 [91-169]</td>
<td>122 [90-163]</td>
<td>126 [93-175]</td>
<td>0.503</td>
</tr>
</tbody>
</table>

The table provides a detailed comparison of demographic and clinical characteristics among three tertiles of Galectin-3 levels in CAD patients, including age, gender, risk factors, heart disease indicators, and laboratory test results. Significant differences were observed across tertiles for several variables, indicating potential associations with Galectin-3 expression.
## Supplemental Table III. Past medical history and medications at baseline of the CAD patients classified by Galectin-3 tertiles.

Results are expressed as absolute number (percentage); comparisons across Galectin-3 tertiles were made by \( \chi^2 \). AMI, acute myocardial infarction; Bypass, coronary artery bypass; Hx, history; PTCA, percutaneous transluminal coronary angioplasty; ACE, angiotensin converting enzyme. \( N = 782 \).

<table>
<thead>
<tr>
<th>Variable</th>
<th>1(^\circ) (n=267, 34.2%)</th>
<th>2(^\circ) (n=255, 32.6%)</th>
<th>3(^\circ) (n=260, 33.2%)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical condition/Hx (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hx of stroke</td>
<td>3 (1.1)</td>
<td>5 (2.0)</td>
<td>5 (1.9)</td>
<td>= 0.695</td>
</tr>
<tr>
<td>Hx of AMI</td>
<td>93 (34.7)</td>
<td>108 (42.5)</td>
<td>123 (47.3)</td>
<td>= 0.012</td>
</tr>
<tr>
<td>Hx of Bypass</td>
<td>30 (11.2)</td>
<td>34 (13.3)</td>
<td>26 (10.0)</td>
<td>= 0.496</td>
</tr>
<tr>
<td>Hx of PTCA</td>
<td>31 (11.7)</td>
<td>22 (8.7)</td>
<td>14 (5.4)</td>
<td>= 0.040</td>
</tr>
<tr>
<td><strong>Medications at baseline (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>97 (36.9)</td>
<td>86 (34.0)</td>
<td>94 (36.6)</td>
<td>= 0.756</td>
</tr>
<tr>
<td>Oral antiplatelet agents</td>
<td>216 (82.1)</td>
<td>201 (79.4)</td>
<td>201 (78.2)</td>
<td>= 0.521</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>114 (43.3)</td>
<td>108 (42.7)</td>
<td>95 (37.0)</td>
<td>= 0.269</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>124 (47.1)</td>
<td>102 (40.3)</td>
<td>106 (41.2)</td>
<td>= 0.233</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>96 (36.5)</td>
<td>103 (40.7)</td>
<td>139 (54.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Heparin</td>
<td>56 (21.3)</td>
<td>68 (26.9)</td>
<td>55 (21.4)</td>
<td>= 0.231</td>
</tr>
<tr>
<td>Diuretics</td>
<td>39 (14.8)</td>
<td>62 (24.5)</td>
<td>107 (41.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Digoxin</td>
<td>10 (3.8)</td>
<td>15 (5.9)</td>
<td>31 (12.1)</td>
<td>= 0.001</td>
</tr>
</tbody>
</table>

*Galectin-3 Tertile - CAD patients*
### Supplemental Table IV. Past medical history and medications at baseline of the CAD patients with preserved LVEF classified by Galectin-3 tertiles.

Results are expressed as absolute number (percentage); comparisons across Galectin-3 tertiles were made by $\chi^2$. AMI, acute myocardial infarction; Bypass, coronary artery bypass; Hx, history; PTCA, percutaneous transluminal coronary angioplasty; ACE, angiotensin converting enzyme.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1° (n=199, 33.4%)</th>
<th>2° (n=201, 33.8%)</th>
<th>3° (n=195, 32.8%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical condition/Hx (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hx of stroke</td>
<td>0 (0.0)</td>
<td>5 (2.5)</td>
<td>4 (2.1)</td>
<td>0.097</td>
</tr>
<tr>
<td>Hx of AMI</td>
<td>59 (29.6)</td>
<td>66 (32.8)</td>
<td>71 (36.4)</td>
<td>0.361</td>
</tr>
<tr>
<td>Hx of Bypass</td>
<td>20 (10.1)</td>
<td>26 (12.9)</td>
<td>19 (9.7)</td>
<td>0.535</td>
</tr>
<tr>
<td>Hx of PTCA</td>
<td>23 (11.7)</td>
<td>21 (10.4)</td>
<td>12 (6.2)</td>
<td>0.151</td>
</tr>
<tr>
<td><strong>Medications at baseline (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>70 (36.1)</td>
<td>72 (35.8)</td>
<td>64 (33.0)</td>
<td>0.777</td>
</tr>
<tr>
<td>Oral antiplatelet agents</td>
<td>159 (82.0)</td>
<td>164 (81.6)</td>
<td>158 (81.4)</td>
<td>0.991</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>96 (49.5)</td>
<td>97 (48.3)</td>
<td>86 (44.3)</td>
<td>0.568</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>90 (46.4)</td>
<td>87 (43.3)</td>
<td>84 (43.3)</td>
<td>0.776</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>62 (32.0)</td>
<td>70 (34.8)</td>
<td>93 (47.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>Heparin</td>
<td>43 (22.2)</td>
<td>47 (23.4)</td>
<td>45 (23.2)</td>
<td>0.953</td>
</tr>
<tr>
<td>Diuretics</td>
<td>22 (11.3)</td>
<td>38 (18.9)</td>
<td>58 (29.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Digoxin</td>
<td>5 (2.6)</td>
<td>7 (3.5)</td>
<td>16 (8.2)</td>
<td>0.019</td>
</tr>
</tbody>
</table>
Supplemental Table V. Multiple regression analysis showing no difference between cases lost and those available at follow-up

<table>
<thead>
<tr>
<th>Variables in the model</th>
<th>$\beta$</th>
<th>$P$</th>
<th>Variables in the model</th>
<th>$\beta$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.040</td>
<td>.453</td>
<td>Left Ventricular EF (%)</td>
<td>0.077</td>
<td>.106</td>
</tr>
<tr>
<td>Gender</td>
<td>0.034</td>
<td>.479</td>
<td>Atherosclerotic burden (Duke score)</td>
<td>0.039</td>
<td>.378</td>
</tr>
<tr>
<td>Smoke (0N1Y)</td>
<td>-0.039</td>
<td>.395</td>
<td>Hx of AMI</td>
<td>0.063</td>
<td>.180</td>
</tr>
<tr>
<td>BMI</td>
<td>0.026</td>
<td>.574</td>
<td>Hx of Bypass</td>
<td>-0.065</td>
<td>.123</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.011</td>
<td>.843</td>
<td>Hx of PTCA</td>
<td>-0.041</td>
<td>.317</td>
</tr>
<tr>
<td>Serum K⁺ (mmol/L)</td>
<td>-0.074</td>
<td>.089</td>
<td>Hx of Diabetes</td>
<td>0.021</td>
<td>.712</td>
</tr>
<tr>
<td>Serum Na⁺ (mmol/L)</td>
<td>0.073</td>
<td>.094</td>
<td>Statins</td>
<td>0.041</td>
<td>.339</td>
</tr>
<tr>
<td>Serum Glucose (mmol/L)</td>
<td>0.029</td>
<td>.619</td>
<td>Oral antiplatelet agents</td>
<td>-0.022</td>
<td>.606</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
<td>0.055</td>
<td>.198</td>
<td>Beta-blockers</td>
<td>0.005</td>
<td>.905</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dL)</td>
<td>-0.040</td>
<td>.346</td>
<td>ACE inhibitors</td>
<td>0.003</td>
<td>.948</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>0.055</td>
<td>.200</td>
<td>Heparin</td>
<td>0.017</td>
<td>.714</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>-0.031</td>
<td>.584</td>
<td>Diuretics</td>
<td>-0.018</td>
<td>.697</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>-0.049</td>
<td>.390</td>
<td>Digoxin</td>
<td>0.005</td>
<td>.910</td>
</tr>
<tr>
<td>Gal-3</td>
<td>-0.025</td>
<td>.591</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Supplemental Table VI. Predictors of CV events at Cox regression analysis.**

**Model 1,** adjusted for age; gender; left ventricular ejection fraction; coronary atherosclerotic burden (Duke score); total, HDL and LDL (high and low density lipoprotein, respectively) cholesterol; BMI (body mass index); hypertension; diabetes; serum sodium; serum potassium; eGFR (estimated glomerular filtration rate); homocysteine; history of myocardial infarction, revascularization by PTCA (Percutaneous transluminal coronary angioplasty), peripheral vascular disease; use of ACE-inhibitors, beta-blockers, diuretics, digoxin, heparin. **Model 2,** adjusted for aforementioned variables excluding drug therapy.

CI, confidence interval; HR, hazard ratio; LVEF, left ventricular ejection fraction; s-Na⁺, serum sodium; ACE, angiotensin converting enzyme. P for significance < 0.05; n = 1013.

<table>
<thead>
<tr>
<th></th>
<th>CV Events</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
<td>Wald</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td><strong>Model 1</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (√ increase)</td>
<td>1.22</td>
<td>(1.03-1.45)</td>
<td>5.04</td>
<td>= 0.025</td>
<td></td>
</tr>
<tr>
<td>LVEF (√ increase)</td>
<td>0.80</td>
<td>(0.70-0.91)</td>
<td>11.07</td>
<td>= 0.001</td>
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</tr>
<tr>
<td>CAD Duke Index Score</td>
<td>1.12</td>
<td>(1.04-1.21)</td>
<td>9.86</td>
<td>= 0.002</td>
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</tr>
<tr>
<td>s-K⁺</td>
<td>1.46</td>
<td>(1.01-2.11)</td>
<td>4.14</td>
<td>= 0.042</td>
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<tr>
<td>Tot cholesterol (Ln increase)</td>
<td>0.39</td>
<td>(0.18-0.83)</td>
<td>5.92</td>
<td>= 0.015</td>
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<tr>
<td>Galectin-3 (Ln increase)</td>
<td>1.15</td>
<td>(0.77-1.72)</td>
<td>0.447</td>
<td>= 0.504</td>
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<td><strong>Model 2</strong></td>
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</tr>
<tr>
<td>Age (√ increase)</td>
<td>1.26</td>
<td>(1.06-1.49)</td>
<td>6.99</td>
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<td>LVEF (√ increase)</td>
<td>0.76</td>
<td>(0.67-0.86)</td>
<td>18.83</td>
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<td>CAD Duke Index Score</td>
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<td>(1.02-1.17)</td>
<td>6.70</td>
<td>= 0.010</td>
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<td>s-K⁺</td>
<td>1.43</td>
<td>(1.00-2.05)</td>
<td>3.84</td>
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<tr>
<td>Tot cholesterol (Ln increase)</td>
<td>0.37</td>
<td>(0.17-0.77)</td>
<td>6.95</td>
<td>= 0.008</td>
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<tr>
<td>Galectin-3 (Ln increase)</td>
<td>1.16</td>
<td>(0.78-1.73)</td>
<td>0.507</td>
<td>= 0.477</td>
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### Supplemental Table VII. Predictors of fatal ischemic events at Cox regression analysis.

**Model 1**, adjusted for age; gender; left ventricular ejection fraction; coronary atherosclerotic burden (Duke score); HDL and LDL (high and low density lipoprotein cholesterol, respectively); BMI (body mass index); hypertension; diabetes; serum sodium; serum potassium; eGFR (estimated glomerular filtration rate); homocysteine; history of myocardial infarction, revascularization by PTCA (Percutaneous transluminal coronary angioplasty), peripheral vascular disease; use of ACE-inhibitors, beta-blockers, diuretics, digoxin, heparin. **Model 2**, adjusted for aforementioned variables excluding drug therapy.

CI, confidence interval; HR, hazard ratio; LVEF, left ventricular ejection fraction; s-Na⁺, serum sodium; ACE, angiotensin converting enzyme. P for significance < 0.05; n = 1013.

<table>
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<tr>
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<td>95%CI</td>
<td>Wald</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Age (√ increase)</td>
<td>1.73</td>
<td>(1.28-2.35)</td>
<td>12.43</td>
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<td>s-Na⁺</td>
<td>0.87</td>
<td>(0.77-0.98)</td>
<td>5.00</td>
<td>= 0.025</td>
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<tr>
<td>Digoxin</td>
<td>3.55</td>
<td>(1.58-7.94)</td>
<td>9.38</td>
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<td>ACE inhibitors</td>
<td>2.21</td>
<td>(1.05-4.63)</td>
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<td>Heparin</td>
<td>0.27</td>
<td>(0.08-0.92)</td>
<td>4.40</td>
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<td>Galectin-3 (Ln increase)</td>
<td>2.28</td>
<td>(1.09-4.74)</td>
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<td>Wald</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Age (√ increase)</td>
<td>1.70</td>
<td>(1.29-2.25)</td>
<td>14.09</td>
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<tr>
<td>LVEF (√ increase )</td>
<td>0.70</td>
<td>(0.54-0.91)</td>
<td>6.88</td>
<td>= 0.009</td>
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<tr>
<td>Galectin-3 (Ln increase)</td>
<td>2.03</td>
<td>(1.01-4.06)</td>
<td>3.94</td>
<td>= 0.047</td>
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## Cardiovascular Mortality - CAD pts

<table>
<thead>
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<th>Wald</th>
<th>P =</th>
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<tr>
<td><strong>Model 1</strong></td>
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</tr>
<tr>
<td>Age (√ increase)</td>
<td>1.44</td>
<td>(1.12-1.87)</td>
<td>7.79</td>
<td>= 0.005</td>
</tr>
<tr>
<td>LVEF (√ increase)</td>
<td>0.68</td>
<td>(0.54-0.86)</td>
<td>10.23</td>
<td>= 0.001</td>
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<tr>
<td>CAD Duke Index Score</td>
<td>1.25</td>
<td>(1.04-1.49)</td>
<td>5.71</td>
<td>= 0.017</td>
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<td>History of PTCA</td>
<td>2.43</td>
<td>(1.05-5.65)</td>
<td>4.28</td>
<td>= 0.039</td>
</tr>
<tr>
<td>Digoxin therapy</td>
<td>2.49</td>
<td>(1.21-5.16)</td>
<td>6.06</td>
<td>= 0.014</td>
</tr>
<tr>
<td>Galectin-3 (Ln increase)</td>
<td>1.87</td>
<td>(1.04-3.33)</td>
<td>4.42</td>
<td>= 0.036</td>
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<tr>
<td><strong>Model 2</strong></td>
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<td></td>
</tr>
<tr>
<td>Age (√ increase)</td>
<td>1.55</td>
<td>(1.22-1.96)</td>
<td>13.01</td>
<td>&lt; 0.001</td>
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<tr>
<td>LVEF (√ increase)</td>
<td>0.59</td>
<td>(0.47-0.74)</td>
<td>21.26</td>
<td>&lt; 0.001</td>
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<tr>
<td>Diabetes</td>
<td>1.72</td>
<td>(1.01-2.92)</td>
<td>3.98</td>
<td>= 0.046</td>
</tr>
<tr>
<td>Galectin-3 (1 Ln increase)</td>
<td>1.82</td>
<td>(1.04-3.20)</td>
<td>4.33</td>
<td>= 0.037</td>
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</table>

**Supplemental Table VIII.** Predictors of cardiovascular mortality at Cox regression analysis in CAD patients.

**Model 1,** adjusted for age; gender; left ventricular ejection fraction; coronary atherosclerotic burden (Duke score); HDL and LDL (high and low density lipoprotein cholesterol, respectively); BMI (body mass index); hypertension; diabetes; serum sodium; serum potassium; eGFR (estimated glomerular filtration rate); homocysteine; history of myocardial infarction, revascularization by PTCA, peripheral vascular disease; use of ACE-inhibitors, beta-blockers, diuretics, digoxin, heparin. **Model 2,** adjusted for aforementioned variables excluding drug therapy.

CI, confidence interval; HR, hazard ratio; CAD, coronary artery cardiovascular disease; LVEF, left ventricular ejection fraction, PTCA, percutaneous transluminal coronary angioplasty. P for significance < 0.05; n = 782.
<table>
<thead>
<tr>
<th>Fatal Ischemic Events - CAD pts with LVEF &gt; 50%</th>
<th>HR</th>
<th>95% CI</th>
<th>Wald</th>
<th>P</th>
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<tbody>
<tr>
<td>Model 1</td>
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</tr>
<tr>
<td>History of PTCA</td>
<td>7.94</td>
<td>(2.38-26.32)</td>
<td>11.33</td>
<td>= 0.001</td>
</tr>
<tr>
<td>Digoxin</td>
<td>13.18</td>
<td>(2.72-62.50)</td>
<td>10.28</td>
<td>= 0.001</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>3.88</td>
<td>(1.28-11.77)</td>
<td>5.70</td>
<td>= 0.017</td>
</tr>
<tr>
<td>Galectin-3 (Ln increase)</td>
<td>5.44</td>
<td>(1.86-15.93)</td>
<td>9.53</td>
<td>= 0.002</td>
</tr>
<tr>
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<tr>
<td>History of PTCA</td>
<td>5.99</td>
<td>(2.11-16.95)</td>
<td>11.36</td>
<td>= 0.001</td>
</tr>
<tr>
<td>Galectin-3 (Ln increase)</td>
<td>5.65</td>
<td>(1.96-16.28)</td>
<td>10.29</td>
<td>= 0.001</td>
</tr>
</tbody>
</table>

**Supplemental Table IX. Predictors of fatal ischemic events at Cox regression analysis in CAD patients with preserved ejection fraction.**

**Model 1** adjusted for age; gender; left ventricular ejection fraction; coronary atherosclerotic burden (Duke score); HDL and LDL (high and low density lipoprotein cholesterol, respectively); BMI (body mass index); hypertension; diabetes; serum sodium; serum potassium; eGFR (estimated glomerular filtration rate); homocysteine; history of myocardial infarction, revascularization by PTCA (Percutaneous transluminal coronary angioplasty), peripheral vascular disease; use of ACE-inhibitor/angiotensin-II type-1 receptor blockers, beta-blockers, diuretics, digoxin, heparin. **Model 2**, adjusted for aforementioned variables excluding drug therapy.

CI, confidence interval; HR, hazard ratio. PTCA, percutaneous transluminal coronary angioplasty. P for significance < 0.05; n = 595.
METHODS

Study Design
The GENICA study protocol was approved by the Medical Ethics Committee. For the sake of space it will be herein recalled only briefly, as it was detailed previously.\(^1\) The recruitment, which started in 1999 and ended in 2001 included consecutive White patients referred to the Division of Cardiology of the Cittadella General Hospital for coronary angiography to investigate chest pain and/or coronary artery disease (CAD). Information on medical history, smoking habits, presence/absence of arterial hypertension, diabetes mellitus, dyslipidemia, and current medications was gathered with a staff administered questionnaire.\(^3\) Definitions for body mass index (BMI), smoking status, hypertension, diabetes mellitus, hyperhomocysteinemia, hypercholesterolemia, and hypertriglyceridemia were already reported.\(^2\),\(^4\) Estimated glomerular filtration rate was calculated from serum creatinine according to the MDRD formula.

Study Population
We enrolled consecutive White patients referred for coronary angiography to investigate chest pain and/or suspected CAD (see flow chart in Figure 1). Refusal to participate in the study was the only exclusion criterion. A written consent to participate in the study was obtained from all patients. Of 1273 potentially eligible patients only two denied consent to the study.

Cardiac catheterization and coronary angiography
Cardiac catheterization with measurement of left ventricular ejection fraction (LVEF) and coronary angiography was performed according to standard procedures. The grading of the CAD burden was carried out with the modified Duke Prognostic Index score as described.\(^4\) This score weighs major epicardial coronary arteries with \(\geq\) 50% diameter stenosis and goes from 0 (all major coronary arteries with lesions < 50% diameter stenosis) to 100 (\(\geq\) 95% left main stenosis). It was reported to accurately predict five-year mortality of medically treated patients.\(^5\)

Laboratory measurements
To minimize potential biases due to a circadian variation of biomarker levels patients were studied between 8.30 a.m. and noon. Blood samples were taken before coronary angiography, collected with heparin and sodium EDTA and divided into aliquots for each type of anticoagulant, immediately put on ice, and centrifuged at 3000xg (at 4°C for 10 min), then stored at -20°C. Aliquots in sodium EDTA were stored without any thawing for 13-15 years until assayed. Serum sodium, potassium, blood urea nitrogen, creatinine, glycaemia, total cholesterol, HDL-cholesterol, and triglycerides levels were measured at enrolment with conventional methods from blood drawn the morning of the coronary angiography.

Gal-3 was assayed blindly to the clinical and follow-up data by chemiluminescent micro-particle immunoassay (CMIA) using a commercially available kit (ARCHITECT Galectin-3 assay, Abbott Diagnostics), according
to manufacturer’s specifications. For samples with Gal-3 concentrations between 4.0 and 114.0 ng/ml the within-assay coefficient of variation of this method was <10%.

**Follow-up data**
Patients information at follow-up was gathered blindly to the biochemical profile with a predefined form through review of medical charts for the patients regularly seen at the referral hospital, and through telephone interviews of family doctors, and/or patients, and/or first-degree relatives for those not attending regular follow-up visits.

Endpoints and adjudication
Predetermined primary endpoints were CV deaths and a composite of CV deaths and CV events, including acute coronary syndromes (ACS) and strokes. Secondary endpoints entailed acute myocardial infarction (AMI) and stroke. Moreover, a composite “fatal ischemic event” endpoint that comprised fatal AMI and fatal stroke was examined (post-hoc). The endpoints were defined following the guidelines available at the time of completion of the follow-up as already reported. All events were validated by the adjudication committee (GPR and GM) blinded to patients’ biochemical profile.

Statistical analysis
A Gaussian distribution was achieved by log or square root transformation of skewed variables, including serum triglycerides, HDL- and LDL-cholesterol, age, creatinine, CAD Duke Index score, LVEF, and Gal-3, as appropriate.

We determined beforehand that 760 patients were needed to achieve a power of 80% at a two sided 5% significance level to detect a difference in survival rate between the group with high and low Gal-3, if the true hazard ratio was 1.7. This was based on the assumption that the accrual period was 24 months, the median follow up period was 90 months, and the median survival was 300 months. Assuming that 25% of the patients would be lost at follow-up, a single random number generation (SPSS) was used to randomly select 1013 subjects from those originally recruited in the GENICA study. The Gal-3 measurement was performed blindly with respect to clinical and anthropometric data.

All the analyses were carried out at first in the whole cohort (n=1013). Then, we completed a sensitivity analysis limited to patients with angiographically documented CAD only (n= 782), selected based on the presence of at least an angiographically detectable stenosis in any of the epicardial coronary arteries. Finally, we included patients with documented CAD and preserved (> 50%) LVEF at angiography (n=595).

Exclusion of univariate and multivariate outliers was decided a priori. Standardized z scores were calculated to identify univariate outliers and cases with z scores exceeding |3.29|, which corresponded to a p<0.001, were excluded. Multivariate outliers were also identified using Mahalanobis
distance (assessed by regression analysis), e.g. cases with $\chi^2$ in excess of 32.909 (12 df at $\alpha=0.001$) were removed from downstream analyses according to Tabachnick and Fidell.\(^9\)

Comparison of quantitative variables across groups was done by ANOVA followed by Bonferroni’s post hoc test. Chi-square analysis was used to compare the frequencies of categorical CAD risk factors. Rho Spearman test was carried out to analyze the association between Gal-3 and atherosclerotic burden. To identify variables independently associated with Gal-3 a regression analysis using inclusion and exclusion criteria of 0.05 and 0.10, respectively, was performed. The dependent variables included in the analysis were: age, LVEF, BMI, LDL- and HDL-cholesterol, triglycerides, estimated glomerular filtration rate, homocysteine, gender, CAD Duke Index score, past medical history (hypertension, diabetes, myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA), coronary artery by-pass surgery, peripheral vascular disease), therapy at enrolment (beta-blockers, ACE-inhibitors, diuretics, heparin, digoxin). A backward variable elimination was chosen because it decreases the risk of missing relevant predictor variables.

Standard multiple regression analysis was used beforehand to verify the assumption that cases lost at follow-up did not differ significantly from those available for survival analysis. Cardiovascular events rates (deaths, myocardial infarction, stroke, and cumulative events) were estimated by Kaplan-Meier analysis with the log-rank test. The predictors of CV events were investigated by Cox stepwise (Wald) regression analysis, using inclusion and exclusion criteria of 0.05 and 0.10, respectively. The variables were entered in three blocks: block 1: age, LVEF, Duke CAD score index; block 2: BMI, LDL- and HDL-cholesterol, estimated glomerular filtration rate, homocysteine, sodium, potassium, gender, past medical history (hypertension, diabetes, myocardial infarction, PTCA, coronary artery by-pass surgery, peripheral vascular disease), therapy at enrolment (beta-blockers, ACE-inhibitors, diuretics, heparin, digoxin); block 3: Gal-3 (as a continuous variable). The block 1 covariates meeting the exclusion criteria were removed; those of block 2 were forced in the model to correct for the imbalance of baseline variables across Gal-3 tertiles. The analysis was carried out in 2 different models: the first including all the variables reported in the aforementioned blocks, the second excluding the medical therapy.

The area under the receiver operating characteristic (ROC) curve was used as an estimate of diagnostic accuracy. The Youden Index (YI) (= max (c) [sensitivity (c) + specificity (c) - 1]) was used to identify the Gal-3 threshold value that corresponds to the value of the ROC curve farthest from the identity line. This index represents the optimal cutoff, defined as the value with the best combination of sensitivity and specificity.

Statistical significance was defined as $P < 0.05$ for primary outcomes and $P < 0.01$ for secondary outcomes. SPSS 20 for MAC (SPSS Italy Inc., Bologna, Italy), SAS system (SAS v9.2, SAS Institute, Cary, NC, USA) were used for all analyses.
References


