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Prognostic role of plasma galectin-3 levels in acute coronary syndrome

Slayman Obeid MD[1*], Nooraldaem Yousif MD[1, 2*], Allan Davies MBBS MPH[3*], Ruben Loretz[1], Lanja Saleh PhD[4], David Niederseer MD[1], Husam A. Noor MD[2], Haitham Amin MD[2], François Mach MD[5], Baris Gencer MD[5], Lorenz Räber MD[6], Stephan Windecker MD[6], Christian Templin MD[1], David Nanchen MD[7], Nicolas Rodondi[8], Olivier Muller MD[8], Christian M. Matter MD[1, 9] Arnold von Eckardstein MD[4], and Thomas F. Lüscher MD, FRCP[3, 9]

[1]Department of Cardiology, University Heart Center Zurich, University Hospital Zurich, Switzerland; [2]Mohammed Bin Khalifa Cardiac Centre; Kingdom of Bahrain; [3]Royal Brompton and Harefield Hospitals and Imperial College, London, United Kingdom; [4]Institute of Clinical Chemistry, University Hospital Zurich, Switzerland; [5]Department of Cardiology, Hospital Universitaire de Geneve, Switzerland; [6]Department of Cardiology, University Hospital Bern, Bern, Switzerland; [7]Department of Ambulatory Care and Community Medicine, University of Lausanne; [8]Department of Cardiology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [8]Department of General Medicine, University Hospital Bern, Switzerland and [9]Center for Molecular Cardiology, University of Zurich, Switzerland.

[*] Equally contributed to this work.

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***Address for Correspondence*:**
Thomas F. Lüscher, MD, FRCP, FACC
Professor of Cardiology and Director of
Research, Education & Development
Royal Brompton and Harefield Hospital Trust
and National Heart and Lung Institute, Imperial College
Sidneystreet
London SW3 6NP, United Kingdom
E-Mail: cardio@tomluescher.ch
Homepage: www.tomluescher.ch

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ABSTRACT

Aim: Cystatin C, neutrophil gelatinase-associated lipocalin (NGAL) and galectin-3 have emerged as biomarker candidates to predict cardiovascular outcomes and mortality in the general population as well as in patients with coronary artery or renal disease. However, their predictive role and clinical utility in patients with acute coronary syndromes (ACS) alone or in combination beyond currently used risk scores remains to be determined.

Methods and Results: Cystatin C, NGAL, and galectin-3 were measured in plasmas of 1'832 patients at the time of presentation with ACS requiring percutaneous coronary intervention or coronary artery bypass grafting. The primary outcomes were major adverse cardiac and cerebrovascular events (MACCE) (defined as the composite of all-cause mortality, cerebrovascular events, any repeat revascularization or myocardial infarction) and all-cause mortality after 1 year and occurred in 192 (10.5%) and 78 (4.3%) of patients, respectively. All three biomarkers were increased in those with MACCE compared with those without (p<0.001). However, only galectin-3 (all-cause mortality: HR=1.027 [95%CI(1.011-1.043);p=0.001], MACCE: HR=1.025 [95%CI(1.012-1.037);p<0.001]) but not cystatin C nor NGAL emerged as independent predictors of both MACCE and death. The risks were particularly high in the highest quartile of galectin-3. The integration of galectin-3 into the GRACE score improved the prediction of MACCE and all-cause mortality significantly. The areas under the receiver operator characteristics curves increased from 0.6701 to 0.6932 for MACCE (p = 0.0474) and from 0.804 to 0.8199 for all-cause mortality (p = 0.0197). Finally, we applied Net Reclassification Improvement (NRI) index using different cut-offs for MACCE which showed negative results (for the cut-offs of 5% and 15%, NRI 0.028, P = 0.586, for the

cut-offs of 10% and 20%, NRI 0.072, P = 0.1132 and for the cut-offs of 10% and 30% the NRI is 0.0843, P = 0.077).

Conclusions: In ACS patients, galectin-3 has moderate prognostic accuracy, provides statistically significant incremental value in some, but not all models, and that the magnitude of any improvement would seem of questionable clinical value.

INTRODUCTION

Cardiovascular (CV) diseases remain the number one cause of deaths worldwide with atherosclerosis as its main underlying cause (1). The formation of plaques, particularly those with a soft and destabilizing lipid-rich core provides the substrate for acute coronary syndromes (ACS) and stroke, which due to superimposed thrombosis may lead to vascular obstruction, organ ischemia or death (2).

Risk scores have been developed for a more tailored management of patients with ACS. Indeed, with the advent of effective, but costly novel drugs in secondary prevention, risk scores are even more important to address the remaining risk in those affected (3). The commonly used GRACE score, is based on age, heart rate, systolic blood pressure, renal function, congestive heart failure, ST-deviation, cardiac arrest and cardiac biomarkers (4).

Biomarkers have significantly improved the diagnosis and risk assessment of ACS, in particular cardiac troponin I (cTnI) and T (cTnT), both components of the contractile apparatus of myocytes. (5, 6). Furthermore, B-type natriuretic peptide BNP and its N-terminal NTproBNP are of great clinical value (7). Finally, C-reactive protein (CRP) and white blood cell counts, both markers of inflammation, predict outcome (8-10).

Cystatin C, neutrophil gelatinase-associated lipocalin (NGAL) and galectin-3 have emerged as biomarker candidates to predict cardiovascular outcomes and mortality in the general population as well as in patients with coronary artery or renal disease. However, their predictive role and clinical utility in patients with acute coronary syndromes (ACS) alone or in combination beyond currently used risk scores remains to be determined.

By the use of a multi-marker approach we here explored the prognostic value of cystatin C, NGAL and galectin-3 in a large prospective cohort of patients with ACS managed according to current guidelines and determined the incremental value of these novel biomarkers compared to currently used risk scores.

METHODS

Patient population: 1’832 patients from the SWISS Special Program University Medicine in ACS (SPUM-ACS) cohort were included in the study (SPUM ACS; NCT01000701) with documentation in a central database and biobank. 267 patients with informed consent were excluded either due to failure to analyse the blood in the dedicated biomarker kit or failure of biomarker measurement. Clinical endpoints were censored at last contact date for 32 patients due to no follow up **(supplementary figure 1).** The cohort comprised of those patients presenting to one of four participating Swiss University Hospitals (Zurich, Bern, Lausanne, and Geneva) between December 2009 and October 2012 with a diagnosis of ACS (STEMI, NSTE-ACS or unstable angina) requiring percutaneous coronary intervention (PCI) or coronary artery bypass grafting.

Patient Selection: Included patients had symptoms compatible with angina pectoris (chest pain, dyspnea) of less than 5 days duration and fulfilled at least one of the following criteria: (a) ECG changes such as persistent ST-segment elevation or depression, T-inversion or dynamic ECG changes, new left bundle branch block (LBBB); (b) evidence of positive (predominantly conventional) troponin by local laboratory reference values with a rise and/or fall in serial troponin levels; (c) known coronary artery disease, specified as status after myocardial infarction, previous PCI or newly documented ≥50% stenosis of an epicardial coronary artery during the initial catheterization. Exclusion Criteria were Patients refusal of informed consent to participate in the registry or lack capacity to consent including foreign languages, High probability of non-adherence to follow-up requirements including patients with sever disabilities and Tourists.

Events Adjudication: All-cause mortality included cardiac, vascular and non-cardiovascular causes of death. Cerebrovascular events comprised stroke (any, ischemic, hemorrhagic, unclear etiology) or transient ischemic attack (TIA); Repeat revascularization included any repeat coronary revascularization (target and non-target vessel). Clinically indicated repeat revascularization included any clinically driven repeat coronary revascularization (target and non-target vessel). Myocardial infarction (MI) was defined based on the universal definition including peri-procedural Myocardial infarction in patients with unstable angina. The primary endpoint of our study and adjudicated major adverse cardiovascular and cerebrovascular events (MACCE), defined as the composite of all-cause mortality, cerebrovascular events, any repeat revascularization or myocardial infarction at 1-year. The secondary end points were adjudicated major adverse cardiovascular events (MACE) defined as the composite of cardiac death, clinically indicated revascularization or MI at 1 year. MACCE were adjudicated by an independent committee of experienced cardiologists (Matthias Pfisterer, MD; Lukas Kappenberger, MD and Tiziano Moccetti, MD).

Biomarkers: Cystatin C, NGAL and galectin-3 were measured in serum sample obtained directly after insertion of the sheath, before performing the diagnostic angiography. Although the time between presentation of the patients to angiography may affect the relative values of individual biomarkers this is unlikely to play a major role as STEMI patients were taken to the catheterization laboratory within minutes of presentation and NSTEMI patients underwent angiography almost always within hours of presentation. Concentrations of Galectin-3 as well as neutrophil gelatinase associated lipocalin (NGAL) in plasma were measured by the use of chemiluminescent microparticle immunoassays (CMIA) on the Architect immunoanalyser (Abbott Laboratories,

Wiesbaden, Germany) (11, 12). Cystatin C was measured in plasma by the use of a particle enhanced turbidimetric immunoassay from Gentian on the Architect Ci8000 analyser (Abbott) by Analytica (Zurich, Switzerland) (13). All assays were performed in a central core laboratory (AvE; Institute for Clinical Chemistry, University Hospital Zurich, Switzerland).

Ethics: The study was approved by the local ethical committees (Kantonale Ethikkomission Zürich, Switzerland; project ID EK-1688). All patients gave written informed consent and all data was anonymized and controlled on site by a dedicated study nurse, all in compliance with the Declaration of Helsinki (14) and the current ICH Guidelines for Good Clinical Practice (15,16). Of note Abbott Laboratories (Wiesbaden, Germany) provided the biomarker assays free of charge but had no role in the statistical evaluation of data and the writing of the manuscript.

Statistics: Continuous variables are expressed as mean ± standard deviation (SD) or medians with interquartile ranges, and were compared using one way ANOVA, Student's t-test, Kruskal-Wallis or Mann-Whitney as appropriate. Categorical data are presented as frequency (percentages) and were compared using the Fisher exact or the chi-square test. We performed univariate regression to determine the predictors of the outcome. Significant predictors of the primary outcome in a univariate analysis were included in multivariate regression model. The choice was mainly determined by clinical and statistical relativity to the studied outcome as well as being meaningful in daily practice. The smoothHR package on R was used for Pointwise Nonparametric Estimation of hazard ratio curves for the continuous variable Galectin-3. (Cite: https://doi.org/10.1155/2013/745742). All statistical analyses including logistic

regression were performed with SPSS 22 and differences were considered significant at $\alpha = 0.05$.

RESULTS

Univariate correlations and associations of the three biomarkers with MACCE and all-cause mortality

The primary end points of MACCE and all-cause mortality occurred in 192 (10.5%) and 78 (4.3%) of patients at 1 year, respectively. Clinical and procedural characteristics in patients with and without MACCE are summarized in **Table 1**.

Upon univariate analysis, all three biomarkers were associated with both MACCE and mortality **(Supplementary Appendix Table 1).** In receiver operator characteristics (ROC) curve analysis (**Figure 1**), the prognostic performance of the unadjusted biomarkers did not differ significantly from each other, neither for all-cause mortality ($AUC_{Galectin\text{-}3}$ = 0.751 vs. $AUC_{Cystatin\ C}$ = 0.761 vs. AUC_{NGAL} = 0.721) nor for MACCE ($AUC_{Galectin\text{-}3}$ = 0.622 vs. $AUC_{Cystatin\ C}$ = 0.632 vs. AUC_{NGAL} = 0.592).

Predictive Value of Galectin-3 for Adverse Cardiac Events and All-cause Mortality

Upon regression analysis, the three markers correlated significantly with each other and by similar strength: r^2 (cystatin C vs. NGAL) = 0.517, r^2 (cystatin C vs. galectin-3) = 0.491), and r^2 (galectin-3 vs NGAL) = 0.497 (acceptable collinearity VIF <5, tolerance >0.2). Also, serum creatinine levels correlated strongly with all three markers, the strongest being cystatin C with r^2 = 0.583 ($p<0.001$).

Galectin-3 but neither cystatin C nor NGAL are independent prognostic biomarkers towards MACCE and all-cause mortality

Multivariate Cox regression model analysis was used to assess the predictors of MACCE and all-cause mortality. After adjusting for age, troponin, myocardial infarction, CABG, history of malignancy, heart failure with reduced ejection fraction (EF<50%),

insulin-dependent diabetes mellitus, resuscitation, galectin-3, NGAL, cystatin C and GFR, the only significant and independent predictors of both MACCE and all-cause mortality during one year of follow-up were age, insulin-treated diabetes mellitus, a history of malignancy, and galectin-3. In addition troponin T was an independent predictor of death (**Table 2**). The hazard ratios of galectin-3 with the one-year-risks of MACCE and all-cause mortality amounted to 1.025 [95%CI(1.012-1.037)]; p<0.001) and 1.027 [95%CI(1.011-1.043)] p = 0.001), respectively. Neither Cystatin C nor NGAL were independent predictors of MACCE (HR=0.814 [95%CI(0.518-1.279] and HR = 1.001 [95%CI(0.998-1.005], respectively) or all-cause mortality (HR = 0.919 [95%CI(0.557-1.519] and HR=1.003 [95%CI(0.999-1.008], respectively) (**Table 2**).

Detailed prognostic properties of galectin-3

The analysis of separate spline curves for hazard ratios showed the presence of a nonlinear, dose-response relationship between serum levels of galectin-3 and risk of both MACCE and all-cause mortality (**figure 2).** To further substantiate our findings, patients were subdivided into quartiles according to the plasma galectin-3 concentrations (Q1:<11.5 ng/ml, Q2:11.5-14.3ng/ml, Q3:14.3-18.3ng/ml, Q4>18.3) and evaluated for the incidence of MACCE and death per quartile (**Table 3)**. In Q4, there was a significantly (p<0.001) higher number of MACCE as well as deaths compared to the lower three quartiles (MACCE: Q4 16.8% vs Q3 10%vs Q2 8.7% vs Q1 6.5% and all-cause mortality: Q4 10.2% vs Q3 3.6% vs Q2 2.4% vs Q1 1.1%). The cumulative survival rate in galectin-3 in the Q4 group was significantly lower than in the Q1, Q2 and Q3 groups (p<0.001) as seen in the Kaplan Meier curve analysis (**Figure 3)**.

We assessed the incremental predictive value of galectin 3 in addition to the GRACE score. For MACCE, Galectin 3 significantly improved the AUC of the ROC

curve from 0.6701 (95% asymptotic CI 0.614 – 0.726) to 0.6932 (0.636 – 0.750) (p = 0.0474, **(Figure 4a)**. For all cause mortality, Galectin 3 significantly improved the AUC from 0.804 (95% CI 0.749 – 0.858) to 0.820 (0.766 – 0.874), p = 0.0197 (Figure 4b).

Finally, we applied Net Reclassification Improvement (NRI) index using two risk cut-offs of 5% and 15% for MACCE, showed a NRI of 0.02822 (std error 0.05194), P = 0.58688 (also for cut-offs of 10% and 20% the NRI is 0.0723, P = 0.1132 and cut-offs of 10% and 30% the NRI is 0.0843, P = 0.07745).

DISCUSSION

The most important finding of our study is that [1] We demonstrated in a multi-marker strategy that out of three renal biomarkers evaluated in this large prospective cohort of patients with ACS, only galectin-3, but not cystatin C or NGAL provided independent, but only modest incremental prognostic information for MACCE and repeat revascularization in some, but not all models (taking into account the negative result obtained from NRI analysis). As such, galectin-3 on its own is not a promising biomarker for further personalized guidance of therapy in patients with ACS. However, in the context of a risk algorithm as artificial intelligence is now about to provide, it may contribute to a more personalized risk assessment. [2] Particularly high galectin-3 plasma levels were associated with a higher incidence of the aggregated endpoint encompassing repeat angina or ACS, arrhythmia or cardiac arrest, congestive heart failure, stroke and cardiac or cerebrovascular death compared to low galectin-3 levels. [3] Finally, MACCE increased with increasing galectin-3 levels in a non-linear fashion. Thus, particularly patients in the upper quartile of galectin-3 levels exhibited a markedly increased MACCE rate and mortality and thus a reduced long-term survival.

Several studies, mostly with low patient numbers, have studied the role of galectin-3 alone, rather than in comparison with other novel markers as investigated in this study. In a small and short-term case control study with 196 STEMI patients and 30 healthy controls galectin-3 levels were higher in STEMI patients than healthy controls and those with high galectin-3 had a higher 30-day MACE and mortality rate (17). However, although ROC analysis suggested galectin-3 levels ≥7.67 ng/mL to be a predictor of 30-day MACE, the sensitivity and specificity were relatively low with 74.5% and 72.4%, respectively (18). Similarly, in another underpowered study of 433 patients with STEMI, NSTEMI, stable CAD and healthy controls high galectin-3 concentrations

were associated with an increased risk of all-cause mortality (19,20). In contrast, another small retrospective study involving only 112 patients with STEMI, previous infarction and controls could not confirm these findings (21). Similarly, Martin-Reyes et al. evaluating 270 patients with prior ACS concluded that galectin-3 was not an independent predictor of MACE (22). While these small and in part retrospective single-centre studies yielded contradictory results, our large prospective multicentre registry with independent and blinded adjudication and a central core lab clearly establishes galectin-3 as an independent predictor of outcome in ACS patients.

There are several possible explanations for the prognostic value of galectin-3. First, galectin-3 destabilizes atherosclerotic plaques as it propagates inflammation, attracts monocytes and macrophages and promotes the formation of foam cells, which secrete galectin-3 and further activate macrophages (32-25). Galectin-3 also dedifferentiates vascular smooth muscle cells, further contributing to plaque vulnerability and ACS (26, 27) In apolipoprotein-E deficient mice, inhibition of galectin-3 not only reduces plaque volume and M_2 macrophage activation, but also plaques (28). This has been confirmed in Apo-E-Galectin-3 double-knockout mice in which not only inflammation and adventitial infiltrates were reduced, but also atheromatous plaques (29). Second, as patients with high galectin-3 exhibit more inflammation they also develop bigger infarcts and in turn lower LVEF. Indeed, three smaller studies found an inverse relation between LVEF and galectin-3 levels in ACS patient (19, 30-32). This may facilitate the development of heart failure during follow-up in those with high galectin-3 levels. Indeed, galectin-3 predicts heart failure hospitalisations and mortality (33). This may be related to the fact that galectin-3 promotes myocardial cardiac fibrosis, thus resulting in maladaptive cardiac remodelling. As galectin-3 is strongly associated with GFR it may be involved in both cardiac and renal dysfunction (34, 35) Overall, this

suggests that galectin-3 is not only a potent predictor of outcome, but also a potential therapeutic target. Blockade of galectin-3 in patients after ACS might not only reverse the atherosclerotic process, but also slow down the development and progression of heart failure.

Cystatin C is a protease inhibitor and its main function is to protect cells from hydrolysis by endogenous and exogenous proteases (36). As such, cystatin C is not only a marker of GFR, but also predicts outcomes in CAD or ACS. Indeed, in 605 ACS patients, Sun et al. found significantly higher plasma cystatin C levels in patients with MACE even after adjustment for confounding factors (37). Although we could confirm these findings, cystatin C did not remain an independent predictor of MACCE and all-cause mortality after adjusting for confounders. Thus, our results obtained in a large prospective cohort dispute these previous studies (38 – 40) and dispute the usefulness of cystatin C in ACS patients. In line with our results, Jernberg et al. comparing cystatin C, creatinine and GFR found that cystatin C was superior in seperating survivors and non-survivors at 35 months. However, after adjustment for variables associated with outcome, cystatin C was also no longer an independent predictor of outcomes (41).

Neutrophil gelatinase-associated lipocalin or NGAL is a 25kD acute phase glycoprotein and a marker of tubular damage and acute kidney injury. NGAL is covalently bound to neutrophil gelatinase in neutrophils and modulates matrix metalloproteinase-9 (MMP-9) activity by preventing degradation and thus preserving its enzymatic activity (42,43). MMP-9 destabilizes atherosclerotic plaques and is involved in tissue repair and vascular remodelling (44). As such serum NGAL is elevated in CAD and correlated with the disease severity (45). In a study of 673 STEMI by Helanova et al. BNP outperformed NGAL for the prediction of heart failure hospitalisation and inclusion of NGAL and BNP to a modified TIMI risk score even improved risk stratification

for mortality (46). In contrast our larger prospective study considering more variables and biomarkers currently used in daily practice and with considerably more statistical power could not confirm these findings. Thus, the prognostic value of NGAL in ACS patients is rather limited.

The strengths of this study are its size providing proper statistical power, its prospective and multicenter design and the fact that an independent committee of experienced cardiologists adjudicated the events and that a certified core laboratory measured the evaluated markers.

CONCLUSIONS

In ACS patients, galectin-3 has moderate prognostic accuracy, provides statistically significant incremental value in some, but not all models, and that the magnitude of any improvement would seem of questionable clinical value.

LIMITATIONS

We did not evaluate the change in biomarker levels over time during the acute ACS event. Therefore, the optimal timing for measuring cystatin C, NGAL and galectin-3 during hospitalization remains to be assessed. Rather we provide baseline values upon presentation immediately before revascularization mainly by primary PCI. It is possible, particularly in STEMI patients, that plasma or serum levels, respectively of these biomarkers change upon revascularization and this may affect their prognostic value. Also our data set for commonly used biomarkers as NT-proBNP and CRP was incomplete; we couldn't analyze the incremental value of galectin-3 when added to GRACE score and NT-proBNP or CRP,

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FIGURES LEGENDS

FIGURE 1. ROC CURVE FOR ALL-CAUSE MORTALITY AS WELL AS MACCE. AUC_{GAL-3} 0.622 VS. $AUC_{CYSTATIN\ C}$ 0.632 VS. AUC_{NGAL} 0.592 FOR ALL-CAUSE MORTALITY. AUC_{GAL-3} 0.751 VS. $AUC_{CYSTATIN\ C}$ 0.761 VS. AUC_{NGAL} 0.721 FOR MACCE. THERE WAS NO SIGNIFICANT DIFFERENCE BETWEEN THESE VALUES.

Figure 2. Smooth hr package Nonparametric estimates of the dependence of all-cause mortality on different Galectin-3 levels among ACS patients (log hazard ratio, with 95% confidence limits). The reference value of Galectin-3 with lowest hazard is 4.9, which corresponds for a probability of zero.

Figure 3. Cumulative survival in patients with different levels of serum Galectin-3 in Kaplan Meier Graph. Q (Quartile)1: <11.5 ng/ml, Q2: 11.5-14.3 ng/ml, Q3: 14.3-18.3 ng/ml, Q4: >18.3 ng/ml. The survival rate in Q4 was significantly lower than in Q1, Q2 and Q3 groups ($p<0.001$).

Figure 4.a. The incremental predictive value of the addition of Galectin 3 to the GRACE score in predicting MACCE. Galectin 3 significantly improved the AUC from 0.6701 (95% asymptotic CI 0.614 – 0.726) to 0.6932 (0.636 – 0.750), p = 0.0474 **Figure 4.b.** The incremental predictive value of the addition of Galectin 3 to the GRACE score in predicting all-cause mortality. Galectin 3 significantly improved the AUC from 0.804 (95% CI 0.749 – 0.858) to 0.820 (0.766 – 0.874), p = 0.0197 .

Supplementary Figure 1. Study flow. The flow diagram shows patient enrollment and follow-up throughout the study. **T1** signifies blood drawing performed at coronary angiography. ***** Not analysed with biomarker kit or failed Measurement. **ACS:** acute coronary syndrome.

TABLES LEGENDS

Table 1. Clinical and procedural characteristics in patients with and without MACCE. (Percentage within MACCE after one year)

Table 2. Hazard ratios and confidence interval for predictors of major adverse cardiac and cerebrovascular Events (MACCE) as well as all-cause mortality

Supplementary Table 1: Results of univariate logistic regression assessing the relationship between each biomarker and major adverse cardiac and cerebrovascular events mortality at one year

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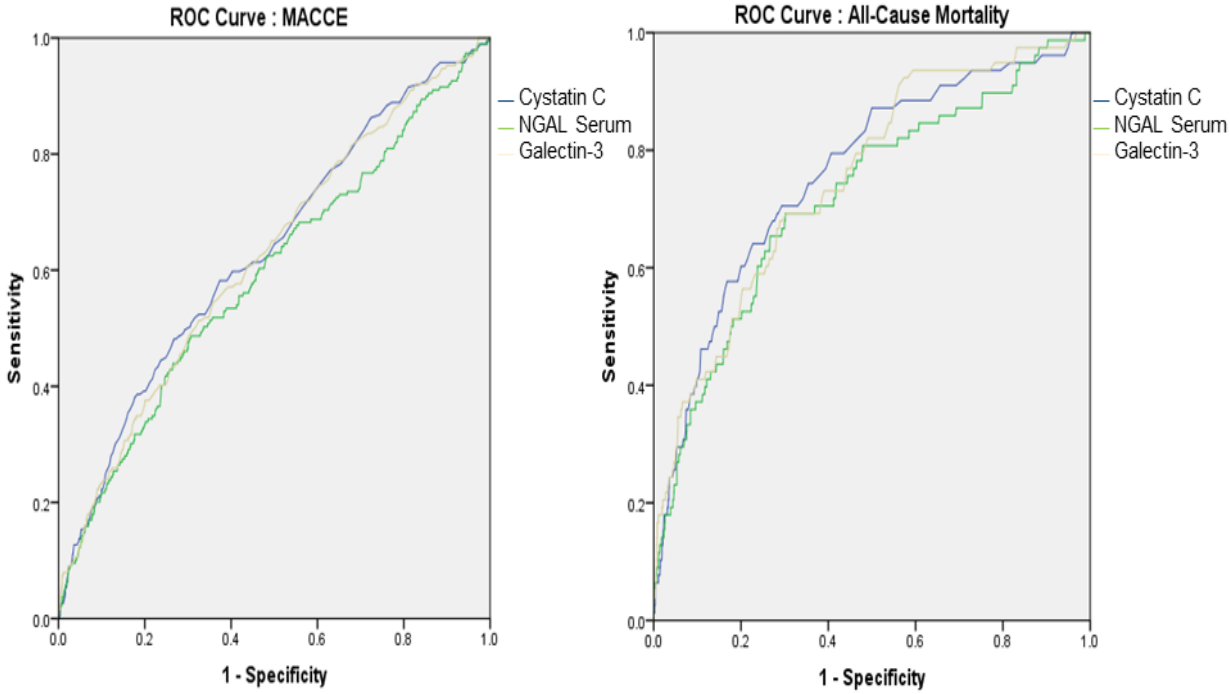

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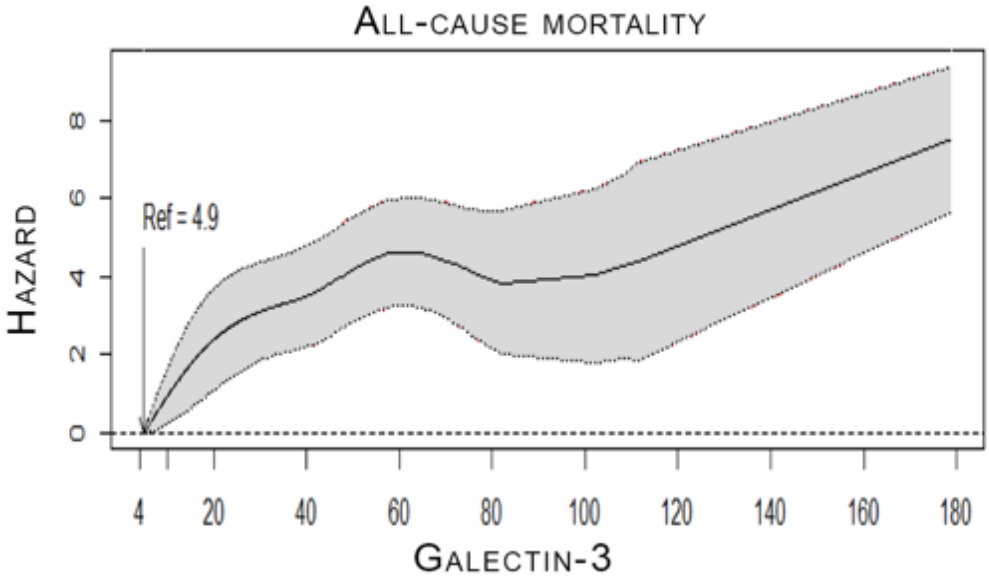

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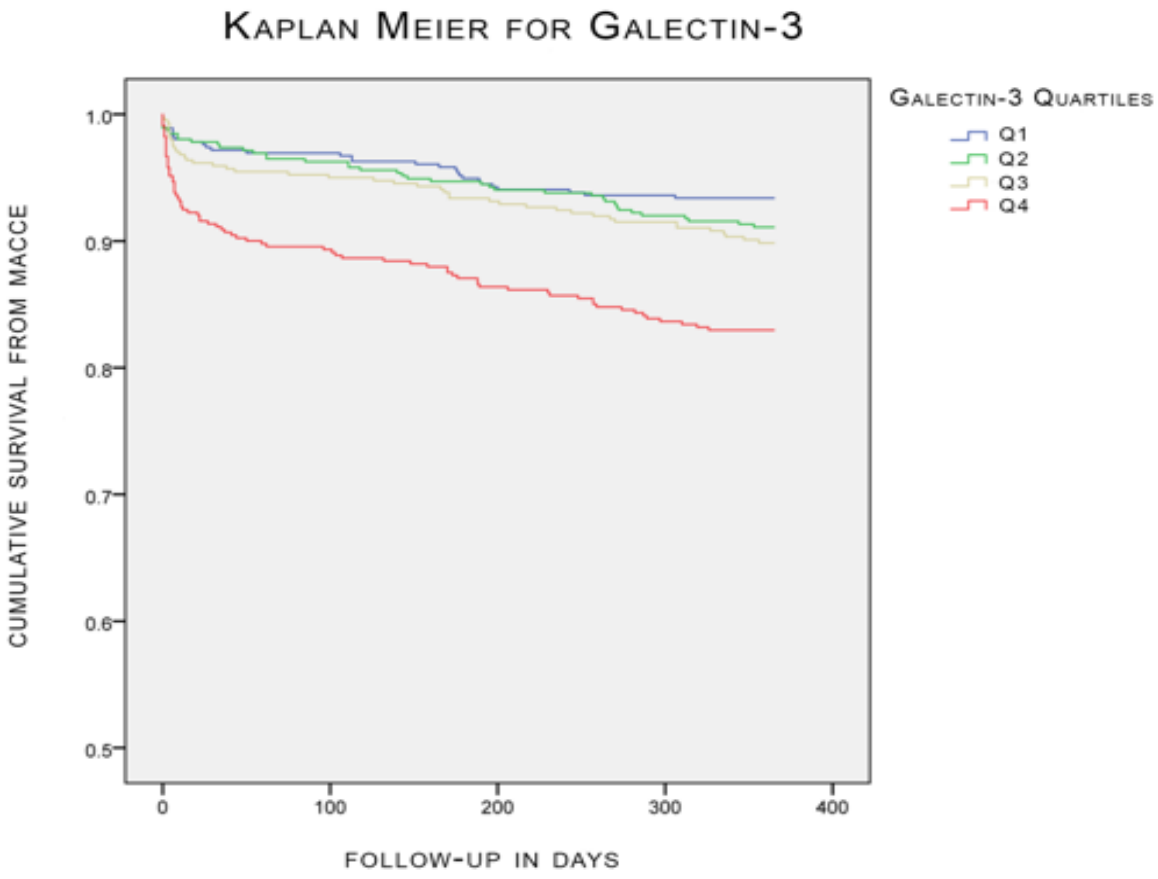

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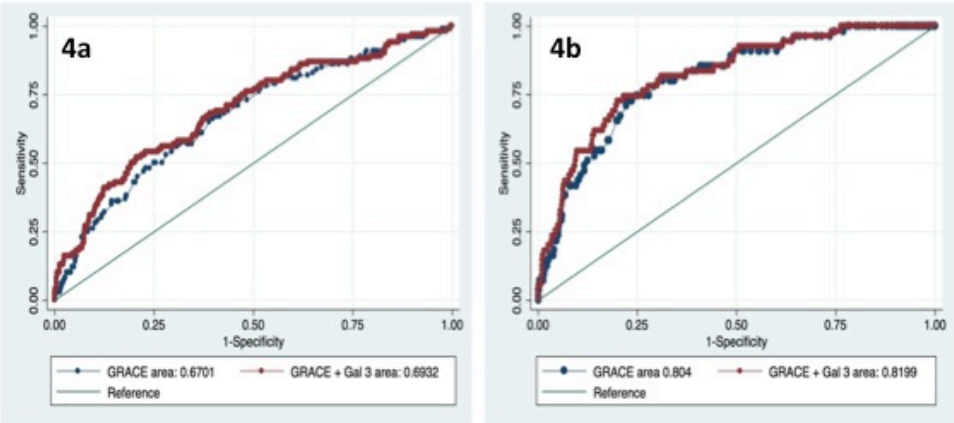
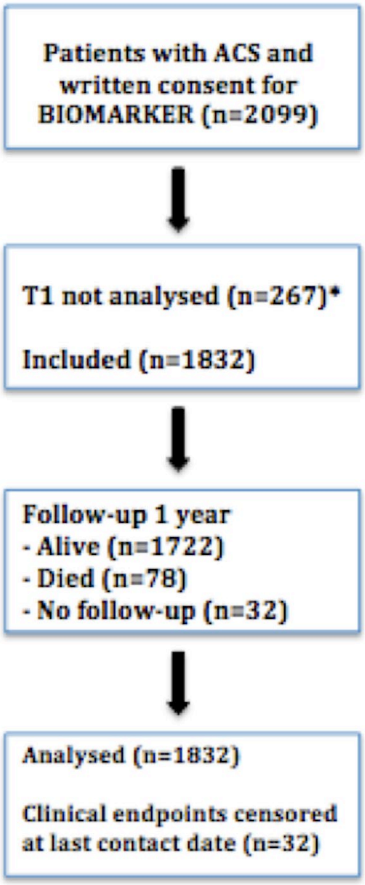


Table 1. Clinical and procedural characteristics in patients with and without MACCE. (Percentage within MACCE after one year)

	MACCE yes	**MACCE no**	***p*-value**
Sex female	41 (21.4%)	341 (20.8%)	0.851
Coronary artery disease	41 (21.7%)	417 (25.8%)	0.251
Peripheral vascular disease	16 (8.3%)	82 (5.0%)	0.061
Diabetes	59 (30.7%)	278 (17.0%)	<0.001
Insulin dependent diabetes mellitus	26 (13.5%)	68 (4.2%)	<0.001
Hypertension	128 (66.7%)	932 (56.8%)	0.011
Cholesterolemia	116 (60.4%)	1013 (61.8%)	0.754
Myocardial infarction	46 (24.0%)	219 (13.4%)	<0.001
PCI history	54 (28.3%)	252 (15.4%)	<0.001
CABG history	24 (12.5%)	68 (4.1%)	<0.001
Congestive heart failure	8 (4.2%)	17 (1.0%)	0.003
Dialysis	2 (1.0%)	6 (0.4%)	0.201
Stroke	5 (2.6%)	33 (2.0%)	0.589
Malignancy	30 (15.6%)	111 (6.8%)	<0.001
Lung disease	14 (7.3%)	67 (4.1%)	0.060
Smoker	123 (65.8%)	1126 (69.5%)	0.316
ACS			0.497
Unstable angina pectoris	9 (4.7%)	62 (3.8%)	
Non STEMI	89 (46.4%)	710 (43.3%)	
STEMI	94 (49.0%)	868 (52.9%)	
Onset			0.747
<24h	123 (64.7%)	1095 (67.2%)	
>24-48h	34 (17.9%)	258 (15.8%)	
>48-72h	20 (10.5%)	141 (8.7%)	
>72h-7d	11 (5.8%)	104 (6.4%)	
>7d	2 (1.1%)	32 (2.0%)	
Resuscitation	8 (4.2%)	49 (3.0%)	0.377
KILLIP score 2 or higher	45 (23.8%)	194 (11.9%)	<0.001
Intraaortic balloon pump	12 (6.3%)	61 (3.7%)	0.115
Vasopressors	8 (4.2%)	32 (2.0%)	0.062

Table 2. Hazard ratios and confidence interval for predictors of major adverse cardiac and cerebrovascular Events (MACCE) as well as all-cause mortality.

	MACCE					**all-cause Mortality**			
	Hazard	95% Confidence Interval		*p*-value		Hazard	95% Confidence Interval		*p*-value
		Lower	Upper				Lower	Upper	
Troponin	1.045	0.978	1.117	0.194		1.124	1.013	1.248	0.028
Age	1.034	1.015	1.053	<0.001		1.083	1.048	1.120	<0.001
MI history	1.419	0.957	2.105	0.082		1.417	0.779	2.577	0.254
CABG	1.617	0.966	2.705	0.067		0.925	0.401	2.132	0.854
Congestive heart failure	1.820	0.866	3.823	0.114		1.784	0.624	5.099	0.280
Malignancy	1.722	1.114	2.661	0.015		2.668	1.505	4.727	0.001
Resuscitation	0.581	0.190	1.778	0.342		0.806	0.205	3.171	0.757
IDDM	2.548	1.631	3.982	<0.001		2.374	1.233	4.568	0.010
Galectin-3	1.025	1.012	1.037	<0.001		1.027	1.011	1.043	0.001
Cystatin C	0.814	0.518	1.279	0.372		0.919	0.557	1.519	0.743
Serum NGAL	1.001	0.998	1.005	0.478		1.003	0.999	1.008	0.156
Clearance	1.003	0.996	1.009	0.405		1.005	0.994	1.017	0.354



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Supplementary Table 1

Results of univariate logistic regression assessing the relationship between each biomarker and major adverse cardiac and cerebrovascular events as well as mortality at one year

Variable	Odds Ratio (95% CI)	P value
MACCE		
Cystatin C (per mg/L increase)	2.01 (1.52-2.68)	< 0.001
NGAL (per ng/ml increase)	1.0056 (1.0033-1.0081)	< 0.001
Galectin-3 (per ng/ml increase)	1.039 (1.026-1.052)	< 0.001
MORTALITY		
Cystatin C (per mg/L increase)	2.99 (2.06 – 4.33)	< 0.001
NGAL (per ng/ml increase)	1.0103 (1.0072 – 1.0134)	< 0.001
Galectin-3 (per ng/ml increase)	1.057 (1.041 – 1.072)	< 0.001