

Cystatin C for Risk Stratification in Patients After an Acute Coronary Syndrome

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Background—Cystatin C (Cys-C) is a marker of renal function that has shown prognostic value for cardiovascular risk stratification across different patient populations. The incremental value of Cys-C beyond established cardiac and renal biomarkers remains incompletely explored.

Methods and Results—SOLID-TIMI 52 (Stabilization of Plaques Using Darapladib-Thrombolysis in Myocardial Infarction 52; www.clinicaltrials.gov, NCT01000727) randomized patients ≤ 30 days post-acute coronary syndrome were treated with darapladib or placebo. The association between Cys-C and long-term risk (median follow-up 2.5 years) was assessed in 4965 individuals with adjustments made for clinical variables and other risk markers (eg, estimated glomerular filtration rate, high-sensitivity troponin I, brain-type natriuretic peptide, and fibroblast growth factor-23). The prespecified outcome of interest was cardiovascular death (CVD) or heart failure hospitalization. Cys-C was strongly correlated with creatinine ($r=0.60$) and estimated glomerular filtration rate ($r=-0.68$), moderately correlated with fibroblast growth factor-23 ($r=0.39$), and weakly correlated with brain-type natriuretic peptide ($r=0.28$) and high-sensitivity troponin I ($r=0.06$) (all $P<0.0001$). After multivariate adjustment, increasing concentration of Cys-C (per SD of log-transformed Cys-C) was significantly associated with a 28% higher hazard of CVD or heart failure hospitalization (hazard ratio [HR] 1.28, 95% confidence interval [CI] 1.12-1.46, $P<0.001$), including CVD (HR 1.24, 95% CI 1.04-1.47, $P=0.01$) and heart failure hospitalization (HR 1.42, 95% CI 1.19-1.69, $P<0.001$). Cys-C was also associated with a higher hazard of CVD, myocardial infarction, or stroke (HR 1.15, 95% CI 1.04-1.28, $P<0.01$), including myocardial infarction (HR 1.17, 95% CI 1.02-1.33, $P=0.02$). The addition of Cys-C to a fully adjusted model without estimated glomerular filtration rate improved the C-statistic from 0.80 to 0.81 ($P=0.03$) for CVD or heart failure hospitalization. In contrast, the addition of estimated glomerular filtration rate to a fully adjusted model without Cys-C failed to improve model discrimination ($P=0.17$).

Conclusions—Cys-C is associated with the risk of adverse outcomes in patients after acute coronary syndrome. This relationship is independent of established and novel biomarkers of the cardiorenal axis. (*J Am Heart Assoc.* 2018;7:e009077. DOI: 10.1161/JAHA.118.009077.)

Key Words: acute coronary syndrome • biomarker • cystatin C • prognosis

Cystatin-C (Cys-C) is a cysteine protease inhibitor produced by almost all human cells. It is excreted into the bloodstream, filtered in the renal glomerulus, and metabolized

by the proximal tubule.^{1,2} Both creatinine and Cys-C are serum measures of renal function, but unlike creatinine, Cys-C is not affected by age, sex, and lean muscle mass and may be more sensitive for detecting mild to moderate changes in estimated glomerular filtration rate (eGFR).^{3,4} Renal dysfunction, as measured by eGFR, has been associated with an increased risk of death among healthy elders,⁵ those with heart failure,⁶ and in stable patients with coronary artery disease,⁷ although the mechanisms remain incompletely defined.

Prior studies have demonstrated an association between Cys-C and the risk of death, including cardiovascular death (CVD) and incident heart failure among ambulatory people with coronary heart disease.⁸ In addition, studies have shown an association between Cys-C and the risk of adverse outcomes after acute coronary syndrome (ACS).⁹⁻¹² However, the incremental value of assessing Cys-C beyond established and novel markers that reflect renal function, including eGFR

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Clinical Perspective

What Is New?

- We investigated the prognostic utility of cystatin C (Cys-C) in 4965 patients after acute coronary syndrome in the SOLID-TIMI 52 (Stabilization of Plaques Using Darapladib-Thrombolysis in Myocardial Infarction 52) trial.
- We found that Cys-C was significantly associated with an increased risk of adverse cardiovascular events independent of established risk factors.
- In addition, we demonstrated that Cys-C provides incremental information for risk stratification independent of traditional and novel biomarkers of the cardiorenal axis including estimated glomerular filtration rate, high-sensitivity troponin I, brain-type natriuretic peptide, and fibroblast growth factor-23.

What Are the Clinical Implications?

- Cys-C helps to identify patients at a greater risk of adverse cardiovascular events after an acute coronary syndrome.
- Cys-C may be a better cardiovascular risk marker in populations with acute coronary syndrome than traditional measures of renal function including creatinine and estimated glomerular filtration rate.

and fibroblast growth factor-23 (FGF-23), remains disputed. Further, its independent prognostic value beyond other cardiovascular markers such as high-sensitivity troponin (hsTn) remains unknown. Thus, we sought to investigate the independent prognostic utility of Cys-C in patients after ACS.

Methods

The data, analytic methods, and study materials are available to other researchers for purposes of reproducing the results or replicating the procedure through the GlaxoSmithKline Data Sharing program.

Study Population and Procedures

The SOLID-TIMI 52 (Stabilization of Plaques Using Darapladib-Thrombolysis in Myocardial Infarction 52; www.clinicaltrials.gov, NCT01000727) study was a double-blind, multicenter, phase 3 trial that enrolled 13 026 patients after ACS and randomized them to receive darapladib 160 mg daily (an inhibitor of lipoprotein-associated phospholipase A₂ activity) or placebo at 868 sites in 36 countries. Detailed descriptions of both the study design and results have been previously published.^{13,14}

In brief, patients were considered potentially eligible for enrollment if they had been hospitalized with an ACS in the

30 days before randomization (unstable angina, non-ST-elevation myocardial infarction [MI], and ST-elevation MI) and had at least 1 of the following high-risk predictors of cardiovascular risk: age ≥ 60 , history of documented MI before qualifying ACS event, diabetes mellitus requiring pharmacotherapy, significant renal dysfunction (defined as eGFR ≥ 30 and ≤ 59 mL/min per 1.73 m² according to the Modification of Diet in Renal Disease Study equation), or established polyvascular disease. A random population of 5000 patients (excluding patients from China, whose samples could not be shipped) was identified before database lock and represented the planned biomarker cohort. Patients with an eGFR < 30 mL/min per 1.73 m² or receiving chronic dialysis before randomization were excluded from study participation.

The study protocol from the original SOLID-TIMI 52 trial was approved by ethics committees at each participating institution, and written informed consent was obtained from all participants. SOLID-TIMI 52 has an active institutional review board approval for continued research with the Partners Human Research Committee.

Laboratory Analysis

At the baseline visit (median 14 days after ACS), samples were obtained and frozen before being shipped on dry ice to be stored at a central lab at -80°C . Biomarker assays were conducted by laboratory personnel blinded to treatment allocation and clinical outcome at the TIMI (Thrombolysis in Myocardial Infarction) Clinical Trials Laboratory (Boston, MA) including Cys-C (Randox assay, Cruclin, UK; Roche Diagnostics c6000 [c501] instrument, Basel, Switzerland); FGF-23 (Immutopics, San Clemente, CA), high-sensitivity troponin I (hsTnI) (Architect i2000SR, Abbott Laboratories, Abbott Park, IL), and brain-type natriuretic peptide (BNP) (Architect i2000SR, Abbott Laboratories, Abbott Park, IL). eGFR was calculated through a central laboratory using the MDRD (Modification of Diet in Renal Disease) Study equation.¹⁵

Study End Points

The prespecified outcome of interest for the current analysis was the composite of CVD or hospitalization for heart failure. Additional outcomes of interest included all-cause mortality, (cardiovascular death, MI, or stroke [MACE]) and its individual components. Cardiovascular end points were adjudicated by a blinded clinical events committee.^{13,14}

Statistical Analyses

Continuous variables were compared with ANOVA with a test for trend. Categorical variables were compared with the Cochran Armitage trend test or chi-squared test. Spearman

correlation was used to assess correlations between markers. Kaplan-Meier event rates are reported at 3 years. Cys-C was prospectively modeled as a log-transformed continuous variable and reported per standard deviation and categorically by quartile. Sensitivity analyses modeled Cys-C as a dichotomous variable (fourth versus first 3 quartiles [Q4:Q1-3]) and eGFR at its clinical threshold of 60 mL/min per 1.73 m². Unadjusted and adjusted Cox proportional hazards models were assessed. Cox regression models were adjusted for clinical predictors of risk including age (quartiles), sex, region (North America and Western Europe versus other regions), race (white versus nonwhite), body mass index (<18.5, 18.5 to <25, 25 to <30, ≥30 kg/m²), smoking status, history of heart failure, diabetes mellitus, hypertension, hyperlipidemia, baseline low-density lipoprotein cholesterol (quartiles), prior MI, index diagnosis (ST-elevation MI versus non-ST-elevation ACS), catheterization for qualifying event, days from qualifying event (≤14 days), eGFR (<60 mL/min per 1.73 m²), BNP (<80 pg/mL), hsTnI (<26 mg/dL), FGF-23 (<93 pg/mL), and randomized treatment arm. The cut point for FGF-23 was 93 pg/mL based on previous analyses in this post-ACS population.¹⁶ Additional adjusted models without FGF-23 and with creatinine rather than eGFR were run.

For the model performance of discrimination and reclassification related to the primary end point (CVD or heart failure [HF]), C-statistics, net reclassification index, and integrated discrimination improvement were calculated in 2 ways: first in a model that contained all variables (including eGFR) for the addition of Cys-C, and second, in a model that included all variables, excluding eGFR and Cys-C, for the addition of the eGFR and Cys-C, separately. All analyses were performed by the TIMI Study Group using the statistical software package SAS version 9.4 (SAS institute, Cary, NC) employing an independent copy of the trial database. A 2-sided $P<0.05$ was considered statistically significant.

Results

Cys-C was available in 4965 patients in the SOLID-TIMI 52 study. The baseline characteristics of the biomarker cohort versus the overall study population are shown in Table S1. Patients with higher concentrations of Cys-C tended to be older, female in gender, and to have a higher body mass index, a history of hypertension, and baseline eGFR <60 mL/min per 1.73 m². Patients with a lower baseline Cys-C concentration were more likely to be smokers, to have been hospitalized with an ST-elevation MI, and more likely to undergo catheterization and percutaneous coronary intervention for the qualifying event. The use of evidence-based therapies including aspirin, P2Y₁₂ inhibitors, and statins was more common in patients with lower Cys-C concentration. Notably,

Cys-C concentration was not significantly associated with race, diabetes mellitus, prior MI, or baseline low-density lipoprotein cholesterol (Table 1).

Cys-C was strongly correlated with creatinine ($r=0.60$, $P<0.0001$) and eGFR ($r=-0.68$, $P<0.0001$), moderately correlated with FGF-23 ($r=0.39$, $P<0.0001$), and weakly correlated with BNP ($r=0.28$, $P<0.0001$) and hsTnI ($r=0.06$, $P<0.0001$).

Association With Outcomes

When modeled as a continuous variable, increasing concentration of Cys-C (per SD of log-transformed Cys-C) was associated with an 89% higher hazard of CVD or HF hospitalization (unadjusted hazard ratio [HR] 1.89, 95% confidence interval [CI] 1.75-2.04, $P<0.001$), including a 91% higher hazard of CVD (HR 1.91, 95% CI 1.79-2.11) and 97% higher hazard of HF hospitalization (HR 1.97, 95% CI 1.78-2.19). Higher Cys-C concentration was also significantly associated with a 44% higher hazard of MACE (unadjusted HR per SD of log-transformed Cys-C 1.44, 95% CI 1.35-1.54, $P<0.001$), including 28% higher hazard of MI (HR 1.28, 95% CI 1.18-1.40).

A stepwise increase in the incidence of CVD or HF and MACE was seen across quartiles of Cys-C (Figures 1 and 2). Patients with Cys-C concentration in the highest quartile had nearly a 5-fold hazard of CVD or HF (HR Q4:Q1 4.87, 95% CI 3.48-6.82, $P<0.001$) and more than a 2-fold hazard of MACE (HR Q4:Q1 2.26, 95% CI 1.82-2.81, $P<0.001$), including HF hospitalization (HR Q4:Q1 5.71, 95% CI 3.58-9.13), CVD (HR Q4:Q1 5.54, 95% CI 3.51-8.77), and MI (HR Q4:Q1 1.72, 95% CI 1.31-2.27) (Table 2).

After multivariable adjustment, the strength of the relationships was attenuated, but increasing concentration of Cys-C (per SD of log-transformed Cys-C) remained significantly associated with a 28% higher hazard of CVD or HF (adjusted HR 1.28, 95% CI 1.12-1.46, $P<0.001$), a 24% higher hazard of CVD (adjusted HR 1.24, 95% CI 1.04-1.47, $P=0.01$), and a 42% higher hazard of HF hospitalization (adjusted HR 1.42, 95% CI 1.19-1.69, $P<0.001$). Cys-C was associated with a 15% higher hazard of CVD, MI, or stroke (adjusted HR 1.15, 95% CI 1.04-1.28, $P<0.01$), including CVD (HR 1.24, 95% CI 1.04-1.47) and MI (HR 1.17, 95% CI 1.02-1.33) (Table 2). When modeling was categorical by quartile, increasing quartiles of Cys-C were associated with a higher hazard of CVD or HF (P -trend=0.01), HF (P -trend≤0.01), and MACE (P -trend=0.045). Consistent results were observed when eGFR was replaced with creatinine in the model (Table S2). Measures of association in the main model excluding FGF-23 are presented in Table S3.

When Cys-C and eGFR were dichotomized (Cys-C Q4:Q1-Q3; eGFR <60 mL/min per 1.73 m²) and entered

Table 1. Baseline Patient Characteristics of the Study Population by Quartile of Baseline Cystatin-C

Characteristic	Quartile 1 (≤0.78 mg/L) (N=1284)	Quartile 2 (0.78-0.88 mg/L) (N=1218)	Quartile 3 (0.88-1.03 mg/L) (N=1246)	Quartile 4 (>1.03 mg/L) (N=1217)	P Trend
Age (y) (median, IQR)	61 (54, 65)	63 (58, 69)	65 (61, 72)	70 (63, 76)	<0.001
Age ≥60 y, n (%)	740 (57.6%)	879 (72.2%)	1021 (81.9%)	1059 (87%)	<0.001
Female, n (%)	284 (22.1%)	312 (25.6%)	292 (23.4%)	396 (32.5%)	<0.001
BMI (kg/m ²), median (IQR)	27.5 (24.8, 30.9)	27.6 (25, 30.1)	27.7 (25.1, 31.2)	28.1 (25, 31.9)	<0.001
Current Smoker, n (%)	277 (21.6%)	215 (17.7%)	248 (19.9%)	176 (14.5%)	<0.001
Race					0.59
White, n (%)	1118 (87.1%)	1079 (88.6%)	1106 (88.8%)	1061 (87.2%)	
Black, n (%)	38 (3.0%)	22 (1.8%)	36 (2.9%)	33 (2.7%)	
Asian, n (%)	106 (8.3%)	96 (7.9%)	89 (7.1%)	103 (8.5%)	
Other, n (%)	22 (1.7%)	21 (1.7%)	15 (1.2%)	20 (1.6%)	
Region					<0.01
North America, n (%)	321 (25%)	285 (23.4%)	258 (20.7%)	265 (21.8%)	
South America, n (%)	82 (6.4%)	90 (7.4%)	120 (9.6%)	108 (8.9%)	
Western Europe, n (%)	415 (32.3%)	350 (28.7%)	394 (31.6%)	332 (27.3%)	
Eastern Europe, n (%)	343 (26.7%)	385 (31.6%)	371 (29.8%)	394 (32.4%)	
Asia Pacific, n (%)	123 (9.6%)	108 (8.9%)	103 (8.3%)	118 (9.7%)	
Hypertension, n (%)	879 (68.5%)	861 (70.7%)	909 (73%)	1029 (84.6%)	<0.001
Hyperlipidemia, n (%)	879 (68.5%)	802 (65.9%)	780 (62.6%)	794 (65.2%)	0.03
Diabetes mellitus, n (%)	488 (38.0%)	384 (31.5%)	357 (28.7%)	444 (36.5%)	0.18
Prior MI, n (%)	423 (32.9%)	353 (29.0%)	387 (31.1%)	407 (33.4%)	0.58
Index event					0.02
Unstable angina, n (%)	147 (11.4%)	136 (11.2%)	164 (13.2%)	150 (12.3%)	
Non-STEMI, n (%)	537 (41.8%)	499 (41%)	516 (41.4%)	565 (46.4%)	
STEMI, n (%)	600 (46.7%)	583 (47.9%)	566 (45.4%)	502 (41.3%)	
ST-segment deviation, n (%)	911 (71.1%)	887 (72.8%)	897 (72%)	834 (68.5%)	0.15
Activities performed for qualifying event					
Catheterization, n (%)	1169 (91.0%)	1056 (86.7%)	1064 (85.4%)	966 (79.4%)	<0.001
PCI, n (%)	1075 (83.7%)	942 (77.3%)	932 (74.8%)	825 (67.8%)	<0.001
Fibrinolytic, n (%)	109 (8.5%)	115 (9.4%)	117 (9.4%)	108 (8.9%)	0.75
Days from qualifying event to randomization, median (IQR)	13 (5, 22)	15 (6, 23)	15 (7, 23)	14 (7, 23)	<0.001
Baseline measurements					
eGFR (mL/min per 1.73 m ²), median (IQR)	96 (84, 108)	84 (78, 96)	78 (66, 84)	60 (48, 72)	<0.001
eGFR<60 (mL/min per 1.73 m ²), n (%)	2 (0.2%)	16 (1.3%)	70 (5.7%)	505 (42.5%)	<0.001
Creatinine (mg/dL), median (IQR)	0.8 (0.7, 0.9)	0.9 (0.8, 1)	1 (0.9, 1.1)	1.2 (1, 1.4)	<0.001
Concomitant medical therapy					
Aspirin, n (%)	1256 (97.8%)	1186 (97.4%)	1198 (96.1%)	1161 (95.4%)	<0.001
P2Y ₁₂ Inhibitor, n (%)	1189 (92.6%)	1085 (89.1%)	1083 (86.9%)	1031 (84.7%)	<0.001
Statin, n (%)	1235 (96.2%)	1160 (95.2%)	1167 (93.7%)	1144 (94%)	<0.01
β-Blocker, n (%)	1123 (87.5%)	1076 (88.3%)	1095 (87.9%)	1071 (88%)	0.77

Continued

Table 1. Continued

Characteristic	Quartile 1 (≤0.78 mg/L) (N=1284)	Quartile 2 (0.78-0.88 mg/L) (N=1218)	Quartile 3 (0.88-1.03 mg/L) (N=1246)	Quartile 4 (>1.03 mg/L) (N=1217)	P Trend
ACE-I or ARB, n (%)	1045 (81.4%)	1013 (83.2%)	1037 (83.2%)	1030 (84.6%)	0.04
Randomized to darapladib, n (%)	640 (49.8%)	606 (49.8%)	624 (50.1%)	614 (50.5%)	0.74

ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; BMI, body mass index; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MI, myocardial infarction; PCI, percutaneous coronary intervention; STEMI, ST-elevation MI.

simultaneously in a model adjusting for other covariates, higher concentrations of Cys-C remained associated with a 34% higher hazard of CVD or HF (adjusted HR Q4:Q1-Q3 1.34, 95% CI 1.01-1.77, $P=0.04$) and an 87% higher hazard of HF (adjusted HR Q4:Q1-Q3 1.87, 95% CI 1.28-2.74, $P<0.001$). In contrast, eGFR was not significantly associated with the hazard of any cardiovascular outcomes (Figure 3).

The addition of Cys-C to a fully adjusted model that excluded eGFR significantly improved the C-statistic (0.80-0.81, $P=0.03$). In contrast, the addition of eGFR to a model that excluded Cys-C failed to improve the C-statistic (0.80-0.80, $P=0.17$; Table 3). The addition of Cys-C to a fully adjusted model that included eGFR had a borderline effect to improve the C-statistic (0.80-0.81, $P=0.052$). Integrated discrimination improvement and net reclassification index are presented in Table S4.

Discussion

We have demonstrated in a large population of patients after ACS that Cys-C is associated with the risk of cardiovascular

outcomes independent of traditional predictors including eGFR. Further, Cys-C provides incremental information for risk stratification that is independent of additional traditional and novel biomarkers of the cardiorenal axis including BNP, hsTnI, and FGF-23.

Interest in Cys-C as a marker of risk first arose when studies demonstrated that it was associated with an increased risk of all-cause mortality, CVD, MI, and stroke among elders from the Cardiovascular Health Study.¹⁷ Later studies suggested a strong association with the risk of death, but not with MI, among patients with stable coronary heart disease⁸; and such results were replicated in post-ACS populations.¹⁸ In brief, Cys-C is a protease inhibitor produced at a constant rate by most human cells; it is filtered by the renal glomerulus, and, unlike creatinine, it is metabolized by the proximal tubule.^{1,2} Thereby, it is a sensitive measure of renal function that may be less affected by age, sex, and lean muscle mass than creatinine^{19,20} and offers a better estimate of GFR than creatinine and conventional creatinine-based formulas.^{21,22} A few mechanisms directly relating Cys-C to the progression of atherosclerosis had been proposed.²³⁻²⁵

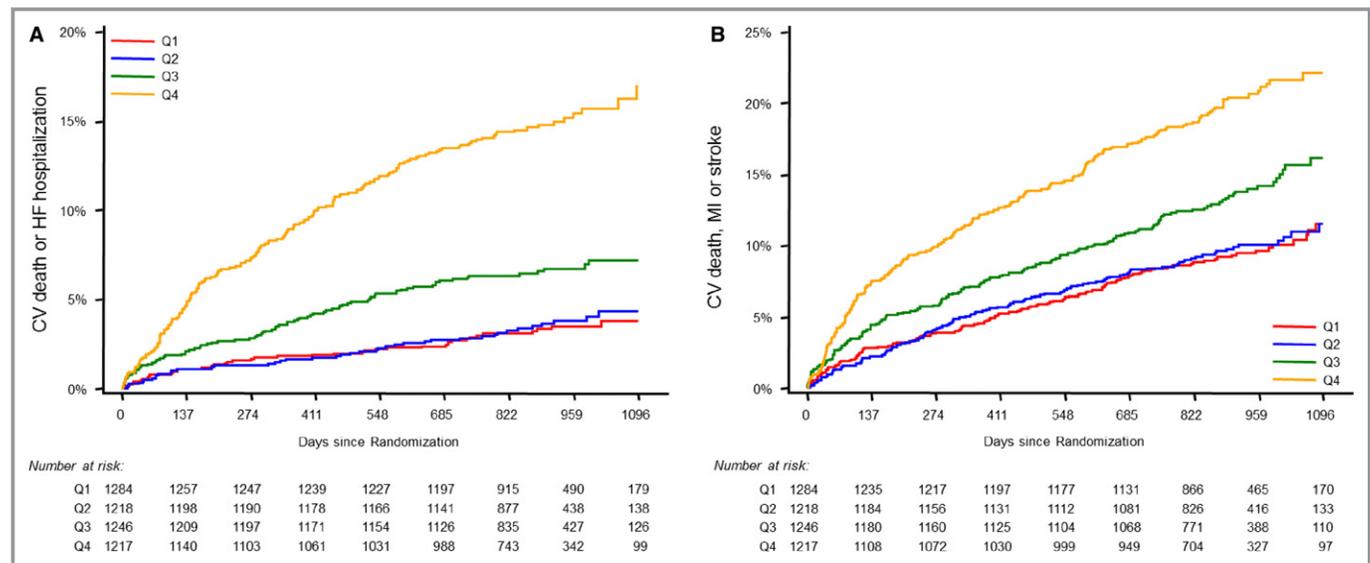


Figure 1. Cumulative incidence curves by cystatin-C quartile through long-term follow-up for the composite end point of cardiovascular death or heart failure hospitalization (A) and MACE (B). CV indicates cardiovascular; HF, heart failure; MACE, major adverse cardiovascular events; MI, myocardial infarction; Q1-Q4, quartiles 1-4.

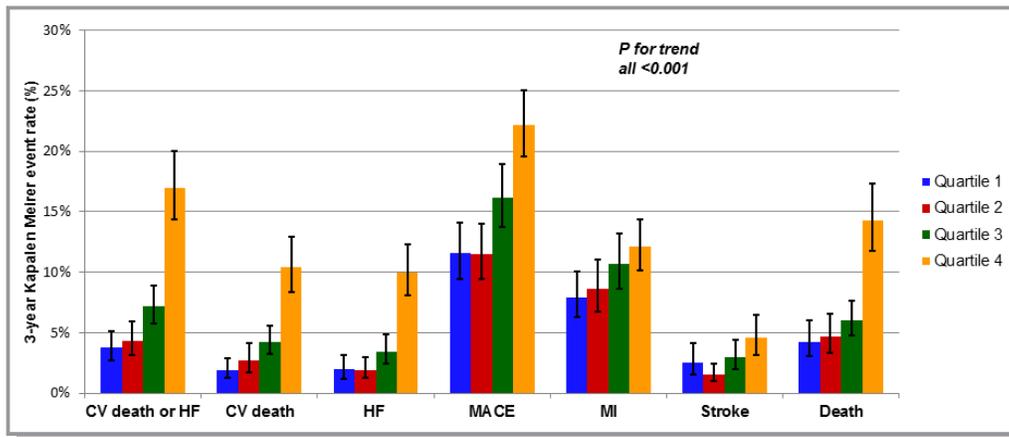


Figure 2. The 3-year Kaplan-Meier event rates of various prespecified outcomes by quartile of baseline cystatin-C. CV indicates cardiovascular; HF, heart failure; MACE, major adverse cardiovascular events; MI, myocardial infarction.

However, a more recent Mendelian randomization study does not support a causal role of Cys-C in the etiology of cardiovascular disease.²⁶

The incremental value of Cys-C beyond simpler assessments of renal function such as eGFR has been often disputed, given the strong correlation between these methods

of assessment. However, these 2 markers may indicate different biological processes. Although both creatinine and Cys-C, are excreted by the kidney, only Cys-C is metabolized by the kidney and thus does not reflect only clearance. In fact, a newer formula for estimating GFR combining creatinine and Cys-C has shown better performance than equations based on

Table 2. Unadjusted and Adjusted Risk of Outcomes by Quartile of Baseline Cystatin-C

Outcome	Number of Events	Model	HR and 95% CI per 1 SD of Log Transformed cystatin-C	P Value	Cystatin-C Quartile				P Trend
					Q1 Adj HR (95% CI)	Q2 Adj HR (95% CI)	Q3 Adj HR (95% CI)	Q4 Adj HR (95% CI)	
CVD or HF	347	Unadjusted	1.89 (1.75–2.04)	<0.001	Referent	1.10 (0.72–1.68)	2.09 (1.44–3.03)	4.87 (3.48–6.82)	<0.001
		Adjusted	1.28 (1.12–1.46)	<0.001	Referent	0.84 (0.54–1.3)	1.32 (0.89–1.96)	1.48 (0.98–2.24)	0.01
CVD	206	Unadjusted	1.91 (1.73–2.11)	<0.001	Referent	1.20 (0.68–2.12)	2.39 (1.45–3.94)	5.54 (3.51–8.77)	<0.001
		Adjusted	1.24 (1.04–1.47)	0.01	Referent	0.84 (0.47–1.52)	1.26 (0.74–2.15)	1.36 (0.78–2.37)	0.14
Hospitalization for heart ure	186	Unadjusted	1.97 (1.78–2.19)	<0.001	Referent	1.10 (0.61–2.01)	1.91 (1.12–3.25)	5.71 (3.58–9.13)	<0.001
		Adjusted	1.42 (1.19–1.69)	<0.001	Referent	0.85 (0.45–1.59)	1.35 (0.77–2.36)	2.08 (1.19–3.66)	<0.01
MACE	651	Unadjusted	1.44 (1.35–1.54)	<0.001	Referent	1.05 (0.81–1.35)	1.48 (1.17–1.87)	2.26 (1.82–2.81)	<0.001
		Adjusted	1.15 (1.04–1.28)	<0.01	Referent	0.95 (0.73–1.24)	1.21 (0.95–1.55)	1.25 (0.94–1.65)	0.045
MI (fatal or nonfatal)	406	Unadjusted	1.28 (1.18–1.4)	<0.001	Referent	1.08 (0.80–1.46)	1.33 (1.00–1.77)	1.72 (1.31–2.27)	<0.001
		Adjusted	1.17 (1.02–1.33)	0.02	Referent	1.15 (0.84–1.56)	1.31 (0.97–1.78)	1.34 (0.94–1.91)	0.08
Stroke (fatal or nonfatal)	116	Unadjusted	1.36 (1.16–1.60)	<0.001	Referent	0.83 (0.45–1.53)	1.38 (0.80–2.38)	2.23 (1.35–3.68)	<0.001
		Adjusted	0.94 (0.74–1.19)	0.59	Referent	0.58 (0.3–1.11)	0.95 (0.54–1.69)	0.97 (0.52–1.81)	0.70
All-cause mortality	303	Unadjusted	1.76 (1.61–1.91)	<0.001	Referent	1.09 (0.71–1.66)	1.74 (1.19–2.54)	3.85 (2.74–5.41)	<0.001
		Adjusted	1.15 (0.99–1.33)	0.06	Referent	0.8 (0.52–1.23)	0.96 (0.64–1.43)	1.05 (0.69–1.6)	0.64

Variables included in the adjusted model are age (quartiles), sex, BMI (<18.5, 18.5–<25, 25–<30, ≥30), history of heart failure, diabetes mellitus, hypertension, hyperlipidemia, prior MI, current smoker, region (North America and Western Europe vs other regions), race (white vs nonwhite), index diagnosis (STEMI vs non-STE ACS), catheterization for qualifying event, baseline LDL cholesterol (quartiles), days from qualifying event (≤14 days), randomized treatment arm, baseline Cys-C, baseline eGFR (<60 mL/min per 1.73 m²), hsTnI (<26 mg/dL), BNP (<80 pg/mL), FGF-23 (<93 pg/mL). BMI indicates body mass index; BNP, brain-type natriuretic peptide; CI, confidence interval; Cys-C, cystatin C; CVD, cardiovascular death; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; HF, heart failure; HR, hazard ratio; hsTnI, high-sensitivity troponin I; LDL, low-density lipoprotein; MACE, major adverse cardiovascular events; MI, myocardial infarction; non-STE ACS, non-ST-elevation acute coronary syndrome; STEMI, ST-elevation MI.

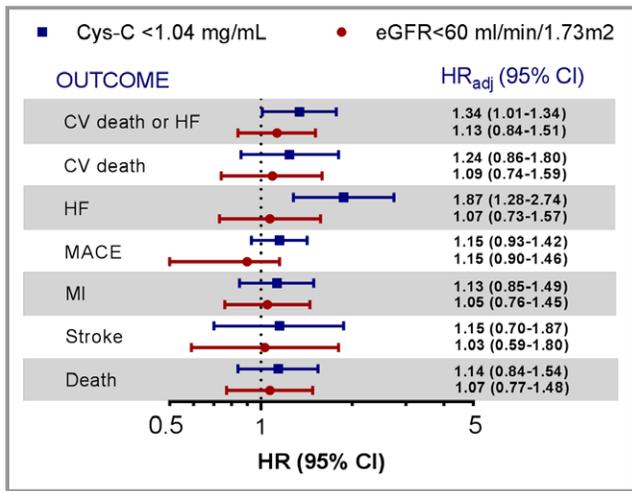


Figure 3. Adjusted risk of outcomes for cystatin-C and eGFR when both are modeled as dichotomous variables and included simultaneously in a model with other risk predictors. Variables included in the model are age (quartiles), sex, BMI (<18.5, 18.5 to <25, 25 to <30, ≥30), history of HF, diabetes mellitus, hypertension, hyperlipidemia, prior MI, current smoker, region (North America and Western Europe vs other regions), race (white vs nonwhite), index diagnosis (STEMI vs non-STE ACS), catheterization for qualifying event, baseline LDL cholesterol (quartiles), days from qualifying event (≤14 days), randomized treatment arm, hsTnI (<26 mg/dL), BNP (<80 pg/mL). BMI, body mass index; BNP, brain-type natriuretic peptide; CI, confidence interval; CV, cardiovascular; Cys-C, cystatin-C; eGFR, estimated glomerular filtration rate; HF, heart failure; HR, hazard ratio; hsTnI, high-sensitivity troponin I; LDL, low-density lipoprotein; MACE, major adverse cardiovascular events; MI, myocardial infarction; non-STE ACS, non-ST-elevation acute coronary syndrome; STEMI, ST-elevation MI.

1 sole marker.²⁷ Earlier studies have shown that Cys-C concentration is strongly associated with long-term mortality in a representative and multiethnic US population with normal renal function²⁸ as well as being associated with the risk of death and cardiovascular disease among elders without chronic kidney disease. It is also predictive of all-cause mortality and CVD among nondiabetics with stage 3 or 4

chronic kidney disease.²⁹ Therefore, it is notable that in our data set, we did not observe a significant association between eGFR and cardiovascular risk once Cys-C was included in the model, yet Cys-C remained independently associated with outcomes.

In the current large-scale study of nearly 5000 patients after ACS, we found an association between Cys-C and the risk of CV death or HF and its individual components, independent of other cardiorenal markers. The current findings add to earlier studies in patients after ACS that showed an association between Cys-C and the risk of death, CVD, and hospitalization for HF.^{9-11,30-32} Although a few small studies have shown a weak association between Cys-C and subsequent MI,^{11,33} larger studies have failed to demonstrate a significant relationship.^{18,34} Cys-C was associated with the risk of MI in PLATO (Platelet Inhibition and Patient Outcome Study) after adjusting for clinical covariates, but this association was no longer significant when established biomarkers were considered. In the current study Cys-C remained associated with the risk of MI after adjustment for clinical predictors and markers, although the relationship was not as strong as that observed with the risk of CVD or HF. Further, there was no apparent relationship between Cys-C and the risk of stroke. Nonetheless, Cys-C added incremental value for cardiovascular risk stratification independent of emerging markers including FGF-23, a marker that is strongly correlated with renal function and the risk of cardiovascular outcomes.³⁵⁻³⁷ To the best of our knowledge, this is the first study that has had the opportunity to evaluate the incremental value of Cys-C after ACS beyond novel prognostic markers such as FGF-23 and hsTnI.

Limitations to our study require consideration. First, despite thorough adjustment for both clinical and biochemical variables, residual confounding cannot be excluded given the observational nature of the analysis. Second, the current observed cut points for Cys-C would require validation in a separate data set before being considered for clinical use. Third, the temporal association and the effects of changes in biomarker concentrations over time were not assessable due to the absence of repeated measures for Cys-C. Last, because

Table 3. Discrimination for Cardiovascular Death or Heart Failure Hospitalization With or Without Cys-C or eGFR

Model	C-Statistic (95% CI)		P Value
	Adjusted Model for Clinical Covariates and Biomarkers (Excluding eGFR and Cys-C)	Adjusted Model Plus New Biomarker	
Adjusted model*±Cys-C	0.80 (0.78–0.82)	0.81 (0.78–0.83)	0.03
Adjusted model*±eGFR		0.80 (0.78–0.83)	0.17

Variables included in the model were age (quartiles), sex, BMI (<18.5, 18.5 to <25, 25 to <30, ≥30), history of heart failure, diabetes mellitus, hypertension, hyperlipidemia, prior MI, current smoker, region (North America and Western Europe vs other regions), race (white vs nonwhite), index diagnosis (STEMI vs non-STE ACS), catheterization for qualifying event, baseline LDL cholesterol (quartiles), days from qualifying event (≤14 days), randomized treatment arm, hsTnI (<26 mg/dL), BNP (<80 pg/mL), FGF-23 (<93 pg/mL). BMI indicates body mass index; BNP, brain-type natriuretic peptide; CI, confidence interval; Cys-C, cystatin-C; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; hsTnI, high-sensitivity troponin I; LDL, low-density lipoprotein; MI, myocardial infarction; non-STE ACS, non-ST-elevation acute coronary syndrome; STEMI, ST-elevation MI.

patients were randomized within 30 days of an ACS (median 14 days), we cannot exclude that the patient's renal function was not at steady state at the time of sample collection, and this may have influenced baseline Cys-C concentration. Consequently, any identified cut points should be validated in additional patient populations. As well, we cannot exclude that the baseline concentration of other assessed biomarkers was influenced by the recent ACS event. The timing from the index event was included as a covariate in all multivariable models but may not have fully captured the variable nature of biomarker fluctuations.

In conclusion, in high-risk patients after ACS, Cys-C is a strong predictor of adverse cardiovascular outcomes, including death from cardiovascular causes and hospitalization for HF; and it provides incremental prognostic information beyond established and novel cardiorenal markers including FGF-23, BNP, creatinine, and eGFR.

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Author Contributions

Correa conducted the literature review and produced the first article draft. Goodrich and Murphy performed statistical analyses. O'Donoghue was the study supervisor and guarantor of this work. All authors interpreted the data and provided critical revision

Disclosures

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SUPPLEMENTAL MATERIAL

Table S1. Baseline patient characteristics for the biomarker cohort compared to the overall study population in SOLID-TIMI 52.

Characteristics	Overall Study Population (N=13,026)		Biomarker population (N=4,965)	
	Value	N	Value	N
Age(yrs) (median, IQR)	64 (59, 71)	13026	64 (59, 71)	4965
Age ≥60 yrs, n (%)	9661 (74.2%)	13026	3699 (74.5%)	4965
Female, n (%)	3326 (25.5%)	13026	1284 (25.9%)	4965
BMI(kg/m ²) (median, IQR)	27.6 (24.8, 31.1)	12964	27.7 (25.0, 31.2)	4951
Current Smoker, n (%)	2472 (19.0%)	13014	916 (18.4%)	4961
Race (pooled)				
White, n (%)	10921 (83.8%)	13026	4364 (87.9%)	4965
Black, n (%)	315 (2.4%)	13026	129 (2.6%)	4965
Asian, n (%)	1573 (12.1%)	13026	394 (7.9%)	4965
Other, n (%)	217 (1.7%)	13026	78 (1.6%)	4965
Region (pooled)				
North America, n (%)	2806 (21.5%)	13026	1129 (22.7%)	4965
South America, n (%)	955 (7.3%)	13026	400 (8.1%)	4965
Western Europe, n (%)	3688 (28.3%)	13026	1491 (30%)	4965
Eastern Europe, n (%)	3773 (29.0%)	13026	1493 (30.1%)	4965
Asia Pacific, n (%)	1804 (13.8%)	13026	452 (9.1%)	4965
Hypertension, n (%)	9555 (73.4%)	13026	3678 (74.1%)	4965
Hyperlipidemia, n (%)	8356 (64.1%)	13024	3255 (65.6%)	4965
Diabetes Mellitus, n (%)	4502 (34.6%)	13026	1673 (33.7%)	4965
Prior MI, n (%)	4046 (31.1%)	13026	1570 (31.6%)	4965
Index Event (Pooled)				
STEMI, n (%)	5883 (45.2%)	13026	2251 (45.3%)	4965
NON-STEMI, n (%)	5559 (42.7%)	13026	2117 (42.6%)	4965
Unstable Angina, n (%)	1584 (12.2%)	13026	597 (12%)	4965
ST-Segment Deviation, n (%)	9227 (70.8%)	13019	3529 (71.1%)	4963
Activities performed for qualifying event				
Catheterization, n (%)	11201 (86.0%)	13025	4255 (85.7%)	4965
PCI, n (%)	9991 (76.7%)	13026	3774 (76%)	4965
Fibrinolytic, n (%)	1203 (9.2%)	13025	449 (9%)	4964
Days from qualifying event to randomization (median, IQR)	14 (6, 23)	13026	14 (6, 23)	4965
Baseline measurements				
eGFR (ml/min/1.73m ²) (median, IQR)	78 (66, 90)	12766	78 (66, 90)	4872
eGFR<60(mL/min/1.73m ²), n (%)	1503 (11.54%)	12766	593 (11.9%)	4872

Creatinine (mg/dL) (median IQR)	0.96 (0.81, 1.10)	12768	1 (0.8,1.1)	4874
Concomitant medical therapy				
Aspirin, n (%)	12559 (96.4%)	13021	4801 (96.7%)	4965
P2Y12 Inhibitor, n (%)	11501 (88.3%)	13021	4388 (88.4%)	4965
Statin, n (%)	12317 (94.6%)	13021	4706 (94.8%)	4965
Beta Blocker, n (%)	11364 (87.2%)	13021	4365 (87.9%)	4965
ACE or ARB, n (%)	10755 (82.6%)	13021	4125 (83.1%)	4965

IQR: interquartile range, BMI: body mass index, MI, myocardial infarction, PCI: percutaneous coronary intervention, eGFR: estimated glomerular filtration rate, ACE-I: angiotensin-converting enzyme inhibitor, ARB: angiotensin-receptor blocker

Table S2. Adjusted risk of outcomes by quartile of cystatin-C after replacing eGFR with creatinine in the main model.

Outcome	Number of events	HR & 95% CI per 1-SD of log transformed cystatin-C	p-value	Cystatin-C quartile				p-trend
				Q1 HR (95%CI)	Q2 HR (95%CI)	Q3 HR (95%CI)	Q4 HR (95%CI)	
CV Death or HF	347	1.30 (1.15-1.48)	<0.001	Referent	0.84 (0.54-1.29)	1.33 (0.90-1.97)	1.52 (1.02-2.27)	<0.01
CV death	206	1.18 (1.00-1.39)	0.06	Referent	0.84 (0.47-1.52)	1.28 (0.75-2.17)	1.3 (0.76-2.25)	0.17
Hospitalization for Heart Failure	186	1.55 (1.31-1.84)	<0.001	Referent	0.85 (0.45-1.58)	1.34 (0.76-2.35)	2.22 (1.28-3.83)	<0.01
MACE	651	1.16 (1.05-1.27)	<0.01	Referent	0.95 (0.73-1.23)	1.22 (0.95-1.56)	1.27 (0.97-1.66)	0.03
MI (fatal or non-fatal)	406	1.18 (1.04-1.33)	0.01	Referent	1.15 (0.84-1.56)	1.31 (0.97-1.78)	1.37 (0.97-1.92)	0.05
Stroke (fatal or non-fatal)	116	0.97 (0.77-1.22)	0.80	Referent	0.58 (0.30-1.11)	0.95 (0.54-1.68)	0.99 (0.541.80)	0.64
All-cause mortality	303	1.15 (1.00-1.32)	0.047	Referent	0.80 (0.52-1.23)	0.96 (0.64-1.44)	1.06 (0.70-1.59)	0.59

Variables included in the model: age (quartiles), sex, BMI (<18.5, 18.5-<25, 25-<30, ≥30), history of heart failure, diabetes mellitus, hypertension, hyperlipidemia, prior MI, current smoker, region (North America and Western Europe vs. other regions), race (white vs. non-white), index diagnosis (STEMI vs. non-STE ACS), catheterization for qualifying event, baseline LDL cholesterol (quartiles), days from qualifying event (≤14 days), randomized treatment arm, baseline Cys-C, baseline creatinine (<1.5 mg/dl), hsTnl (<26mg/dL), BNP (<80 pg.mL), FGF-23 (<93 pg/mL). Of note, collinearity was observed between cystatin C and creatinine when both were combined in the model although the relationship between cystatin-C and CV risk remained significant.

CV: cardiovascular, HF: heart failure, MACE: major adverse cardiovascular events, MI: myocardial infarction.

Table S3. Adjusted risk of outcomes by quartile of baseline cystatin-C, excluding FGF-23.

Outcome	HR & 95% CI per 1-SD of log transformed cystatin-C	p-value	Cystatin-C quartile				p-trend
			Q1 Adj HR (95%CI)	Q2 Adj HR (95%CI)	Q3 Adj HR (95%CI)	Q4 Adj HR (95%CI)	
CV Death or HF	1.46 (1.29-1.65)	<0.001	Referent	0.92 (0.60-1.42)	1.48 (1.00-2.18)	2.05 (1.38-3.05)	<0.001
CV death	1.43 (1.22-1.69)	<0.001	Referent	0.92 (0.51-1.65)	1.43 (0.85-2.43)	1.94 (1.13-3.32)	<0.01
Hospitalization for Heart Failure	1.60 (1.36-1.90)	<0.001	Referent	1.01 (0.55-1.86)	1.51 (0.87-2.64)	2.88 (1.67-4.97)	<0.001
MACE	1.22 (1.11-1.34)	<0.001	Referent	0.99 (0.76-1.28)	1.26 (0.98-1.61)	1.43 (1.09-1.88)	<0.01
MI (fatal or non-fatal)	1.20 (1.06-1.36)	<0.01	Referent	1.16 (0.85-1.58)	1.34 (0.99-1.81)	1.45 (1.03-2.04)	0.02
Stroke (fatal or non-fatal)	1.05 (0.83-1.32)	0.71	Referent	0.64 (0.34-1.22)	1.01 (0.57-1.79)	1.20 (0.65-2.20)	0.31
All-cause mortality	1.31 (1.14-1.50)	<0.001	Referent	0.85 (0.55-1.30)	1.06 (0.71-1.59)	1.41 (0.94-2.13)	0.06

Variables included in the model: age (quartiles), sex, BMI (<18.5, 18.5-<25, 25-<30, ≥30), history of heart failure, diabetes mellitus, hypertension, hyperlipidemia, prior MI, current smoker, region (North America and Western Europe vs. other regions), race (white vs. non-white), index diagnosis (STEMI vs. non-STE ACS), catheterization for qualifying event, baseline LDL cholesterol (quartiles), days from qualifying event (≤14 days), randomized treatment arm, baseline Cys-C, baseline eGFR (<60ml/min/1.73m²), hsTnI (<26mg/dL), BNP (<80 pg/mL).

CV: cardiovascular, HF: heart failure, MACE: major adverse cardiovascular events, MI: myocardial infarction.

Table S4. Reclassification for cardiovascular death or heart failure hospitalization with Cystatin C or eGFR.

Model	Absolute IDI (95% CI)	p-value	Category-Less NRI (95% CI)	p-value
Adjusted model* + Cys-C	0.004 (0.000-0.007)	0.048	0.35 (0.25, 0.46)	<0.001
Adjusted model* + eGFR	0.001 (-0.002-0.004)	0.42	-0.16 (-0.27, -0.054)	<0.01
Adjusted model* (including eGFR) + Cys-C	0.003 (0.000-0.006)	0.07	0.28 (0.17, 0.39)	<0.001

**Variables included in the model: age (quartiles), sex, BMI (<18.5, 18.5-<25, 25-<30, ≥30), history of heart failure, diabetes mellitus, hypertension, hyperlipidemia, prior MI, current smoker, region (North America and Western Europe vs. other regions), race (white vs. non-white), index diagnosis (STEMI vs. non-STE ACS), catheterization for qualifying event, baseline LDL cholesterol (quartiles), days from qualifying event (≤14 days), randomized treatment arm, hsTnl (<26mg/dL), BNP (<80 pg.mL), FGF-23 (<93 pg/mL).*