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ORIGINAL RESEARCH

Circulating Cystatin C Is an Independent Risk Marker for Cardiovascular Outcomes, Development of Renal Impairment, and Long-Term Mortality in Patients With Stable Coronary Heart Disease: The LIPID Study

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BACKGROUND: Elevated plasma cystatin C levels reflect reduced renal function and increased cardiovascular risk. Less is known about whether the increased risk persists long-term or is independent of renal function and other important biomarkers.

METHODS AND RESULTS: Cystatin C and other biomarkers were measured at baseline (in 7863 patients) and 1 year later (in 6106 patients) in participants in the LIPID (Long-Term Intervention with Pravastatin in Ischemic Disease) study, who had a previous acute coronary syndrome. Outcomes were ascertained during the study (median follow-up, 6 years) and long-term (median follow-up, 16 years). Glomerular filtration rate (GFR) was estimated using Chronic Kidney Disease Epidemiology Collaboration equations (first GFR-creatinine, then GFR-creatinine-cystatin C). Over 6 years, in fully adjusted multivariable time-to-event models, with respect to the primary end point of coronary heart disease mortality or nonfatal myocardial infarction, for comparison of Quartile 4 versus 1 of baseline cystatin C, the hazard ratio was 1.37 (95% CI, 1.07–1.74; P=0.01), and for major cardiovascular events was 1.47 (95% CI, 1.19–1.82; P<0.001). Over 16 years, the association of baseline cystatin C with coronary heart disease, cardiovascular, and all-cause mortality persisted (each P<0.001) and remained significant after adjustment for estimated GFR-creatinine-cystatin C. Cystatin C also predicted the development of chronic kidney disease for 6 years (odds ratio, 6.61; 95% CI, 4.28–10.20) independently of estimated GFR-creatinine and other risk factors. However, this association was no longer significant after adjustment for estimated GFR-creatinine-cystatin C.

CONCLUSIONS: Cystatin C independently predicted major cardiovascular events, development of chronic kidney disease, and cardiovascular and all-cause mortality. Prediction of long-term mortality was independent of improved estimation of GFR.

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Chronic kidney disease is among the risk factors for atherothrombotic cardiovascular disease (CVD) which have received increasing attention.[1,2] Its strength as a risk factor increases with advancing age.[3]

Cystatin C, a cysteine protease inhibitor that regulates cathepsin S and K and hence vascular biology, is produced and released from all human nucleated cells at a constant rate. It has a low molecular mass and is freely

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CLINICAL PERSPECTIVE

What Is New?

- Circulating cystatin C level is an independent predictor of major cardiovascular events during 6 years of follow-up in patients with coronary heart disease, after adjustment for conventional risk factors, estimated glomerular filtration rate based on cystatin C, and other important biomarkers.
- Circulating cystatin C level predicts long-term cardiovascular mortality in patients with coronary heart disease during 16 years of follow-up, independent of its ability to better estimate glomerular filtration rate.
- Cystatin C is a significant predictor of development of chronic kidney disease during 6 years of follow-up, independently of estimated glomerular filtration rate-creatinine, and of other risk factors.

What Are the Clinical Implications?

- The findings underscore the importance of chronic kidney disease as a cardiovascular risk factor in older people.
- Circulating cystatin C level has additional prognostic value for major cardiovascular events and long-term mortality beyond improved assessment of estimated glomerular filtration rate using cystatin C.
- Other factors, such as the availability and cost-effectiveness of the assay, should be considered before estimation of cystatin C levels is incorporated into usual clinical practice.

Nonstandard Abbreviations and Acronyms

LIPID	Long-term Intervention with Pravastatin in Ischemic Disease

filtered by the renal glomeruli to be reabsorbed and fully catabolized in the proximal renal tubules. It is not influenced by body habitus, muscle mass, or sex. It was proposed many years ago as a measure of estimated glomerular filtration rate (eGFR)[4] and meta-analyses have shown that blood levels of cystatin C allow a more accurate measure of renal function than serum creatinine.[5] Indeed, based on studies where GFR has been directly measured, eGFR is better estimated from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using both cystatin C and creatinine in the model.[6]

Several population-based studies have shown that elevated plasma cystatin C levels are associated with CVD events and mortality.[7,8] Patients with coronary heart disease (CHD) with elevated cystatin C are also at higher risk after adjustment for traditional risk factors.[9,10] Although some have suggested that the effect of cystatin C on CVD outcomes may be at least partly independent of renal function and could reflect other effects on atherosclerosis, the evidence supporting this is relatively limited.[11,12] There is also a relative lack of data relating cystatin C levels to long-term outcomes,[11,13,14] particularly in patients with CHD.

The blood biobank stored from patients in the LIPID (Long-Term Intervention with Pravastatin in Ischemic Disease) study[15] was used in the present analyses. This allowed robust assessment of the independent prognostic role of plasma cystatin C levels in prediction of CVD outcomes and development of chronic kidney disease during the LIPID study, and cause-specific and all-cause mortality during 16 years of follow-up.

METHODS

The authors declare that all supporting data are available within the article and its online supplementary materials.

LIPID Study Design

The design and results of the LIPID study have been reported previously.[15] Patients were aged 31 to 75 years, had myocardial infarction (MI) or hospitalization for unstable angina 3 to 36 months previously and had baseline total cholesterol of 155 to 271 mg/dL (4.0–7.0 mmol/L) and triglycerides <445 mg/dL (<5.0 mmol/L). They were randomized to pravastatin 40 mg daily or placebo. The primary end point of the LIPID study was CHD mortality. In all, 7863 (87% of the total cohort of 9104 patients) had biomarkers measured from baseline samples.

The study was terminated early on the recommendation of the Data and Safety Monitoring Committee after a median of 6 years follow-up, because the predetermined stopping boundary for efficacy had been crossed. At this time, 6889 patients in the total cohort were alive, of whom 6106 had biomarkers measured from blood drawn 12 months after randomization.

At closure, the trial results were disseminated to all patients and their treating medical practitioners, and 6754 patients then commenced an open label statin. After this, patients were followed up for a further 10 years (total follow-up, median 16 years), specifically for CVD events by direct follow-up for the initial 2 years, and beyond this for cause-specific and all-cause mortality by linkage to national death registries in Australia and New Zealand. (Figure S1).

The LIPID trial was approved by the ethics committee at each participating center. All patients gave

written informed consent before the study and separately, for prolonged clinic or remote follow-up.

Laboratory Methods and Biomarkers

Blood was drawn into EDTA tubes at baseline before randomization, and 12 months later, after a 12-hour fast. Plasma samples were then stored in freezers at –70 °C.

Cystatin C levels were measured by latex microparticle immunoassay (Abbott Diagnostics, Architect c8000) in the MORGAM (MONICA Multinational Monitoring of Trends and Determinants in Cardiovascular Disease, Risk, Genetics, Archiving, and Monograph) biomarker laboratory.[16] The assay range for cystatin C was 0.0005 to 10.0 mg/dL and the inter-assay coefficient of variation was 1.2%.

Additional biomarkers were also assayed, chosen to reflect the range of pathobiological processes considered important in atherothrombotic disease: B-type natriuretic peptide (BNP, myocardial stress), sensitive troponin I (TnI, myocardial injury), high-sensitivity C-reactive protein (inflammation), lipoprotein-associated phospholipase A2 (plaque instability), mid-regional pro-adrenomedullin (humoral), lipoprotein (a) (lipids), and D-dimer (coagulation).[16,17] Performance of these biomarkers are shown in Table S1.

Cardiovascular and Mortality Outcomes

Analyses for outcomes were pre-specified in a biomarker protocol. The composite of CHD mortality or nonfatal MI after 6 years was pre-specified as the primary outcome for LIPID sub-studies, including those of biomarkers.[17] Other end points assessed in this present analysis included CHD mortality; major cardiovascular events (CVD mortality, nonfatal MI, and stroke); stroke; CVD mortality; and all-cause mortality, as reported previously.[15]

End points for analyses extending up to 16 years of follow-up included cause-specific mortality (CVD mortality, cancer mortality, and non-CVD non-cancer mortality) and all-cause mortality.

All deaths, MIs, and strokes until the end of ≈ 8 years were adjudicated by expert committees of cardiologists and neurologists who were masked to treatment allocation. After that time the cause of death was sourced from death registries. Our previous work showed a high level of agreement of this with adjudicated events[18] but any misclassification would tend to dilute the strength of the associations between cystatin C and the outcome.

Assessment of Renal Function

The CKD-EPI equation uses a 2-slope "spline" to model the relationship between GFR and serum creatinine, age, sex, and ethnicity,[19] and is now the most commonly used to estimate GFR. It was used to assess renal function in the present study at baseline and during follow-up. The development of chronic kidney disease (CKD), using the CKD-EPI creatinine equation, was defined among patients without CKD at baseline (with an eGFR ≥60 mL/min per 1.73 m^2), by them having 2 subsequent eGFR readings <60 mL/min per 1.73 m^2 at the 5-year time point and at study close.[6,20] Patients with significant renal disease as judged by their clinician (usually eGFR <40 mL/min per 1.73 m^2) at baseline assessment were not randomized. In addition, baseline GFR was here estimated using the CKD-EPI creatinine-cystatin C equation[6] in sensitivity analyses. A single spot urine at baseline was tested for proteinuria, but urinary albumin-creatinine ratio was not assessed.

Statistical Analysis

Baseline cystatin C levels were grouped by quartiles, with cut points of ≤0.72, >0.72 to ≤0.81, >0.81 to ≤0.93, >0.93 mg/L. Changes in cystatin C between baseline and 1 year were also grouped by quartiles with cut points of decrease of >0.05, decrease of 0.05 to 0, increase of 0 to 0.04, increase of >0.04 mg/L. Similarly, eGFR and other biomarkers were also grouped and analyzed in quartiles because their associations with outcomes were not linear.

The associations between the median of each quartile of baseline cystatin C levels with demographic and clinical variables were assessed using a linear model for continuous outcomes and logistic regression for binary outcomes. The statistical test in each case was a test for trend over the quartiles of cystatin C levels.

The relationships between quartiles of cystatin C levels and outcomes in the 6-year study period were assessed using pre-specified Cox models, which initially included only study treatment and sex (Adjusted Model 1) and then adjusted for eGFR (estimated using the CKD-EPI creatinine equation)[19] and other cardiovascular risk factors at baseline including age, prior stroke, diabetes, current smoking, hypertension, fasting glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, nature of the qualifying prior acute coronary syndrome, timing of coronary revascularization, systolic blood pressure, atrial fibrillation, body mass index, New York Heart Association dyspnea class, Canadian Cardiovascular Society angina grade, white blood cell count, peripheral vascular disease, use of aspirin, and proteinuria on spot urine test (Adjusted Model 2). The *P* value presented for the effect of cystatin C is a test of trend over the quartiles. When significant this indicates an overall increase in the risk, rather than a strictly linear increase, as the fourth quartile often dominated the risk. Evidence against linearity was assessed[21] and was not significant. The variables included in the models were based on the

independent predictors of cardiovascular events in risk models that had been previously published from analyses of the LIPID data.[22,23] Model 3 also adjusted for the other novel biomarkers assayed. When used, backward selection was performed manually, fitting the model and removing the predictor with the largest *P* value and then repeating this process. This was done to maximize the amount of data available at each assessment because of missing values for some of the baseline risk factors (<1% overall). Additional analyses were undertaken by adjusting for eGFR using the CKD-EPI creatinine-cystatin C equation.[6]

The proportional hazards assumption was tested both graphically and using the cumulative sums of Martingale residuals.[21] The assumption of proportional hazards was met for cystatin C. Event rates were calculated as Kaplan–Meier estimates. In all time-to-event models, the interaction between the intervention and baseline risk factors was assessed in a global fashion and was not significant. The interaction between cystatin C and eGFR was also assessed for all outcomes and was not significant (each $P>0.2$). Interaction between cystatin C and randomized treatment was also examined in a time-to-event model.

For analysis of cause-specific and all-cause mortality to 16 years, similar Cox regression methods were used, but with the following modifications. To meet the assumption of proportional hazards, statin treatment was included in the Cox model partitioned into 2 periods: the 6 years of the randomized controlled trial and the additional 10 years of extended follow-up. These models included the risk factors already specified for analyses within the trial period. To assess whether the strength of the associations with long-term mortality outcomes varied over time the association of cystatin C and deaths was also examined in 5-year bands (0–5, 6–10, and >10 years) by stratifying time in the model.

Discrimination of each risk model was assessed using net reclassification improvement (NRI) with 4 risk categories, and the C statistic.[24] The risk category cut-offs were based on the same levels used in earlier publications.[17] While the C statistics are presented for both the base model and the model with the biomarker added the change was not assessed because of bias in this measure for censored data.[25]

For both the trial period of 6 years and the long-term follow-up to 16 years, NRI was calculated from Kaplan–Meier probabilities for the addition of cystatin C to models containing the usual risk factors.[26,27] The NRIs associated with BNP and TnI, the biomarkers for which there is most evidence about their predictive value for cardiovascular events, were compared with those for cystatin C. The end points assessed were the composite of CHD mortality and nonfatal MI, and CVD mortality in the trial period, and CVD and all-cause mortality during long-term follow-up. To enable comparison with the most important traditional risk factors for future CVD events, the NRI was also calculated in a baseline model with the same standard risk factors except for the variable being assessed, and then with the addition of age and history of MI as the qualifying event for the study, separately.

The relationship between change in cystatin C levels from baseline to 1 year and subsequent outcomes was assessed in landmark analyses in the 6106 patients who survived to 1 year and with biomarkers available at that time point.

These landmark analyses used methods which were similar to those above. The first analysis, after adjusting for baseline levels, examined the impact of change in cystatin C from baseline to 12 months on outcomes to the end of the randomized trial. The second, again after adjusting for baseline cystatin C levels, examined the effect of change in cystatin C on cause-specific and all-cause mortality up to 16 years of total follow-up.

To assess whether cystatin C added information independently of eGFR-creatinine to the risk of developing CKD during the study, data from the subset of 3946 patients without CKD at baseline were analyzed. As time to development of CKD was unavailable, the relationship between cystatin C levels and development of CKD was assessed using logistic regression accounting for eGFR (using the CKP-EPI creatinine equation), proteinuria at baseline, sex, diabetes, hypertension, body surface area, high-density lipoprotein cholesterol, and triglycerides. The odds ratios from these models are conditional on patients surviving to 5 years. We also assessed whether the relationship between baseline cystatin C and new CKD remained after adjustment for both cystatin C and eGFR at 12 months. This relationship was also subsequently assessed after adjustment for eGFR based on CKD-EPI creatinine-cystatin C equation rather than the CKP-EPI creatinine equation.[6]

All analyses were performed on an intention-to-treat basis. Associations are presented as hazard ratios when comparing the highest quartile (4) with the lowest quartile (1) as referent. The 95% CI for the discrimination analyses were calculated by bootstrapping with 1000 replications. *P* values when relating to associations of biomarker levels and outcomes are for the trend across biomarker quartiles. Results were not adjusted for multiple comparisons. Analyses used SAS 9.4 (SAS Institute Inc, Cary, NC, USA). All authors had access to the study data.

RESULTS

Baseline Characteristics and Cystatin C Levels

There were no clinically important differences in baseline risk factors between the 7863 patients with

biomarker measurements and the 1151 without such assays (data not shown).

Table 1 shows that at baseline, patients with higher cystatin C levels were more often older, with a higher proportion of women and lower eGFR. They also more often had other CVD risk factors, including smoking, hypertension, dyslipidemia, diabetes, and obesity, as well as atrial fibrillation, a high white blood cell count, measures of angina and dyspnea, and associated with these various factors, the use of cardiovascular medications. Cystatin C levels were also significantly associated with an overall measure of the risk of recurrent CHD events, either CHD mortality or MI, as estimated by the LIPID risk score ($P<0.001$).[22] There was no significant difference in baseline cystatin C levels between those randomized to pravastatin or placebo.

Cystatin C Levels and Outcomes During the LIPID Study

After adjustment for sex and randomized treatment allocation only, a higher baseline cystatin C level was significantly associated with an increased risk of all pre-specified outcomes of interest during the 6 years of the LIPID study (Table 2, Adjusted Model 1, each P for trend <0.001).

Most of these significant associations of baseline cystatin C levels remained after adjustment for all significant CVD risk factors previously identified in LIPID study analyses, including eGFR and the other novel biomarkers assayed (Table 2, Adjusted Model 3). Baseline cystatin C remained a significant predictor of the composite of CHD mortality and nonfatal MI (HR Q4 versus Q1: 1.37; 95% CI, 1.07–1.74; P for trend=0.01), and also major cardiovascular events (HR 1.47; 95% CI, 1.19–1.82; P for trend <0.001), CVD mortality (HR 1.44; 95% CI, 1.04–1.99; P for trend =0.03), stroke (HR 1.63; 95% CI, 1.02–2.59, P for trend =0.04), and all-cause mortality (HR 1.35; 95% CI, 1.04, 1.75; P for trend =0.02); however, the hazard ratios were reduced. When these analyses were repeated after adjustment for eGFR based on creatinine-cystatin C, plasma cystatin C remained significantly associated with major CVD events (HR, 1.37; 95% CI, 1.05–1.79; P=0.02) but not the other outcomes, though HR estimates were similar (Table S2). By contrast, neither eGFR-creatinine nor eGFR-creatinine-cystatin C remained significant in these models containing plasma cystatin C.

Cystatin C, Pravastatin Treatment, and Clinical Outcomes

The relative reduction in cardiovascular events with pravastatin was similar in each quartile of baseline cystatin C levels (all P for interaction >0.13) but absolute benefits were larger among patients in the higher cystatin C quartiles (Table S3). For the pre-specified primary end point of CHD death or nonfatal MI, in those in the highest quartile of baseline cystatin C levels, the event rate during the 6-year study period was reduced from 21.9% to 19.0% in those randomized to pravastatin (number needed to treat=28).

In those who survived to 12 months, whether randomized to pravastatin or placebo there were minimal changes in cystatin C levels between baseline and 12 months (0.002 and −0.006 mg/L, respectively).

Cystatin C Levels and Mortality During 16 Years of Follow-Up

After adjustment for all significant traditional CVD risk factors including eGFR-creatinine, and for the other novel biomarkers, higher baseline cystatin C levels were a significant and independent predictor of CHD mortality (HR, 1.49; 95% CI, 1.24–1.79), CVD mortality (HR, 1.42; 95% CI, 1.21–1.68), non-CVD non-cancer mortality (HR, 1.86; 95% CI, 1.41–2.45), and all-cause mortality (HR, 1.42; 95% CI, 1.25–1.63) over 16 years (each P for trend <0.001, Table 3 and Figure S2). Cystatin C levels did not predict cancer mortality. In these models, cystatin C, BNP, and TnI all remained strong independent predictors of the outcome, while eGFR-creatinine also remained significant, but the associations were not as strong (Figure S2). When these analyses were repeated after adjustment for eGFR based on CKD-EPI creatinine-cystatin C, plasma cystatin C remained significantly associated with CHD mortality (HR, 1.45; 95% CI, 1.14–1.84; P=0.002), non-CVD non-cancer mortality (HR, 2.37; 95% CI, 1.64–3.41; P<0.001) and total mortality (HR, 1.41; 95% CI, 1.19–1.67; P<0.001) (Table S4).

The ongoing value of cystatin C in predicting long-term mortality outcomes is further illustrated in Table S5. This shows that for all causes of death, the long-term associations remained strong with a continued increased risk of death among survivors beyond 10 years. The results in this time period for Quartile 4 versus 1 were; for CHD mortality (HR, 2.11; 95% CI, 1.64, 2.72; P<0.001), for CVD mortality (HR, 1.97; 95% CI, 1.57–2.46; P<0.001), for cancer mortality (HR, 1.75; 95% CI, 1.24–2.47; P=0.05), for non-CVD non-cancer mortality (HR, 2.06; 95% CI, 1.49–2.84; P<0.001), and for all-cause mortality (HR, 1.92; 95% CI, 1.63–2.25; P<0.001).

Net Reclassification

Net reclassification indices based on 4 pre-specified groups and using methods for time-to-event outcomes[27] for both the randomized study period and long-term follow-up are shown in Table 4. The NRIs associated with cystatin C, BNP, TnI, age, and history of MI had relatively wide CI but were generally similar for the outcomes of the composite of CHD mortality or nonfatal MI, long-term CHD mortality and all-cause mortality.

Table 1. Baseline Characteristics by Cystatin C Quartiles†

	Cystatin C (mg/L)				
	≤0.72	>0.72 to ≤0.81	>0.81 to ≤0.93	>0.93	*P* trend*
No. of subjects	2020	1996	1981	1866	
Cystatin (mg/L); mean (SD)	0.66 (0.05)	0.77 (0.03)	0.87 (0.03)	1.10 (0.19)	
Pravastatin assignment	1000 (50%)	1026 (51%)	1003 (51%)	912 (49%)	0.50
Age at randomization (y); median (IQR)	56 (50–63)	61 (54–66)	64 (58–68)	67 (62–70)	<0.001
Women	326 (16%)	292 (15%)	323 (16%)	392 (21%)	<0.001
Months from qualifying event; median (IQR)	15.3 (8.2–25.3)	13.7 (7.8–24.9)	13.4 (7.9–24.9)	13.4 (7.7–24.9)	0.05
Atrial fibrillation	7 (0%)	18 (1%)	34 (2%)	51 (3%)	<0.001
Current smoker	163 (8%)	187 (9%)	179 (9%)	206 (11%)	0.003
Diabetes	175 (9%)	154 (8%)	144 (7%)	203 (11%)	0.010
Obesity	322 (16%)	332 (17%)	378 (19%)	365 (20%)	<0.001
Previous stroke	52 (3%)	59 (3%)	97 (5%)	114 (6%)	<0.001
Systolic blood pressure (mm Hg); mean (SD)	131 (18)	133 (19)	136 (19)	138 (20)	<0.001
Diastolic blood pressure (mm Hg); mean (SD)	80 (11)	81 (11)	81 (11)	81 (11)	<0.01
Dyspnea NYHA Class>1	135 (7%)	147 (7%)	205 (10%)	274 (15%)	<0.001
Angina CCVS Grade>0	667 (33%)	681 (34%)	763 (39%)	816 (44%)	<0.001
Baseline lipids					
Total cholesterol ≥5.5 mmol/L (212.7 mg/dL)	1192 (59%)	1098 (55%)	1135 (57%)	1070 (57%)	0.66
HDL-c <1 mmol/L (38.7 mg/dL)	1088 (54%)	1255 (63%)	1288 (65%)	1296 (69%)	<0.001
Triglycerides ≥1.5 mmol/L (132.9 mg/dL)	989 (49%)	1061 (53%)	1097 (55%)	1169 (63%)	<0.001
Previous coronary revascularization	812 (40%)	849 (43%)	853 (43%)	739 (40%)	0.59
Qualifying event: Prior MI vs not	1315 (65%)	1263 (63%)	1268 (64%)	1174 (63%)	0.23
Proteinuria in spot urine	79 (4%)	78 (4%)	110 (6%)	160 (9%)	<0.001
Aspirin	1712 (85%)	1680 (84%)	1613 (81%)	1496 (80%)	<0.001
ACE inhibitor	212 (10%)	269 (13%)	306 (15%)	467 (25%)	<0.001
Beta-blocker	886 (44%)	940 (47%)	967 (49%)	898 (48%)	0.008
Calcium antagonist	635 (31%)	621 (31%)	693 (35%)	739 (40%)	<0.001
LIPID risk score; mean (SD)	4.8 (3.3)	5.5 (3.4)	6.1 (3.5)	7.0 (3.4)	<0.001
LIPID risk score; median (IQR)	5.0 (2.0–7.0)	5.0 (3.0–7.0)	6.0 (4.0–8.0)	7.0 (5.0–9.0)	
Baseline biomarker concentrations					
eGFR (mL/min per 1.73 m^2); median (IQR)	80 (70–90)	73 (65–82)	68 (60–77)	57 (50–66)	<0.001
White blood cell count (10^3/μL); median (IQR)	6.7 (5.8–7.9)	6.9 (5.8–8.1)	7.2 (6.2–8.3)	7.2 (6.2–8.5)	<0.001
BNP (pg/mL); median (IQR)	15.3 (6.6–31.3)	21.1 (9.0–42.4)	25.9 (11.5–54.3)	40.1 (16.7–80.6)	<0.001
hs-CRP (mg/L); median (IQR)	1.7 (0.9–3.4)	2.1 (1.1–4.1)	2.7 (1.4–5.1)	3.7 (1.9–6.8)	<0.001
D-dimer (mg/L); median (IQR)	1.8 (0.9–3.4)	2.3 (1.2–4.2)	2.8 (1.4–5.1)	3.3 (1.6–7.0)	<0.001
Sensitive troponin I not detectable	814 (40%)	780 (39%)	779 (39%)	594 (32%)	<0.001

(*Continued*)

Table 1. Continued

	Cystatin C (mg/L)				
	≤0.72	>0.72 to ≤0.81	>0.81 to ≤0.93	>0.93	*P* trend*
Lp(a) (mg/dL); median (IQR)	13.8 (6.3–47.0)	13.5 (6.7–44.3)	14.1 (6.6–43.2)	14.0 (6.8–42.4)	0.33
Mid-regional pro-adrenomedullin (nmol/L); median (IQR)	0.39 (0.32–0.45)	0.45 (0.38–0.51)	0.51 (0.44–0.59)	0.63 (0.52–0.74)	<0.001
Lp-PLA_2 activity (nmol/min per mL); median (IQR)	250 (219–283)	259 (229–289)	265 (236–296)	270 (237–305)	<0.001

No. (%) is presented unless otherwise stated. LIPID Risk Score is derived from Marschner et al., 2001.[22] BNP indicates brain natriuretic peptide; CCVS, Canadian Cardiovascular Society; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; Lp(a), lipoprotein (a); Lp-PLA_2, lipoprotein-associated phospholipase A2; MI, myocardial infarction; and NYHA, New York Heart Association.

**P* values for trend for continuous variables are from a linear model, and for binary variables from a logistic regression.

†Missing data: Previous coronary revascularization (n=28), body mass index (n=1), white blood cell count (n=1), fasting glucose (n=18), proteinuria (n=1).

Overall, BNP was probably the superior biomarker in its discriminative ability, and TnI possibly inferior to cystatin. However, in these patients with CHD, whose mean age at baseline was 62 years, advancing age was by far the best discriminator for long-term mortality outcomes. While the C statistics for the base model and the base model with the biomarker of interest are presented, no statistical comparisons are included because of the bias in these measures for censored data.[25]

Change in Cystatin C Levels and Outcomes

During the LIPID randomized study, after adjustment for other CVD risk factors, including eGFR-creatinine, and baseline levels of cystatin C and other novel biomarkers, an increase in cystatin C levels from baseline to 1 year of >0.04 mg/L was again associated with the risk of CHD mortality or nonfatal MI (HR for Quartile 4 of change, 1.35; 95% CI, 1.12–1.64; *P* for trend =0.002), major CVD events (HR for Quartile 4 of change, 1.37; 95% CI, 1.15–1.62; *P* for trend <0.001), and stroke (HR for Quartile 4 of change, 1.43; 95% CI, 1.02–2.01; *P* for trend =0.03) (Table S6). Change in cystatin C levels was also associated with all-cause mortality (1.27; 95% CI, 1.04–1.55; *P* for trend =0.02) but not with CHD or CVD mortality.

After another 10 years of follow-up, an increase in cystatin C levels between baseline and 1 year was associated with increased risk of CHD mortality (HR, 1.26; 95% CI, 1.08–1.46; *P* for trend 0.004), CVD mortality (HR, 1.31; 95% CI, 1.15–1.50; *P* for trend <0.001), non- CVD non-cancer mortality (HR, 1.43; 95% CI, 1.13–1.83; *P* for trend 0.002), and all-cause mortality (HR, 1.27; 95% CI, 1.15–1.41; *P*<0.001). There was no association with cancer mortality (Table S7).

Cystatin C and Deterioration in Renal Function

At trial close, 385 of the 3946 patients with a normal eGFR at baseline had developed CKD (defined as an eGFR- creatinine <60 mL/min per 1.73 m^2 at both year 5 and study close). As shown in Figure S3, baseline levels of both cystatin C and eGFR-creatinine were independent predictors of the development of CKD. When considering the effect of baseline cystatin C levels alone, the odds of developing CKD among those in Quartile 4 compared with Quartile 1 was 12.0 (7.9–18.2). This effect was attenuated when eGFR-creatinine was added to the model, but remained high, with odds ratio (OR)=6.3 (4.1–9.7). The relationship between baseline cystatin C levels and development of CKD was similar after further adjustment for other known risk factors for CKD (sex, diabetes, proteinuria at baseline, hypertension, and body surface area; OR, 6.6; 4.3–10.2). In addition, the significant association of baseline cystatin C with development of CKD (data not shown) remained so after adjustment for both baseline and year 1 measures of eGFR-creatinine (*P*<0.001).

To determine whether cystatin C better predicts development of CKD because of its ability to better measure baseline renal function or by other means, we also evaluated the eGFR-cystatin C-creatinine equation in the model (Figure S3). This showed that eGFR creatinine-cystatin C was a significant prognostic factor for development of CKD (HR, 0.02 [0.01–0.07]; *P*<0.001) independently of eGFR-creatinine; while circulating cystatin C did not significantly improve the prediction when added to a model containing eGFR creatinine-cystatin C (HR, 1.25 [0.73–2.13], *P*=0.45).

DISCUSSION

In patients who were stable after a previous acute coronary syndrome, we comprehensively evaluated the role of circulating cystatin C levels and eGFR, the conventional measure of renal function derived using the CKD-EPI equation and creatinine levels, as prognostic markers for the risk of further cardiovascular and renal outcomes, and long-term mortality. Analyses adjusted for a broad range of important clinical and

Table 2. Risk of 6-Year Cardiovascular End Points by Baseline Cystatin C Levels, Unadjusted and Adjusted for Other Risk Factors

End point cystatin C mg/L	Events/total	5-y event rate (%)	Model 1, adjusted for only sex and treatment*		Model 2, adjusted for standard demographic and clinical risk factors†,‡		Model 3, adjusted for standard demographic, clinical risk factors, and all novel biomarkers‡,§	
			HR (95% CI)	*P* Value‖	HR (95% CI)	*P* Value‖	HR (95% CI)	*P* Value‖
CHD events (CHD mortality and nonfatal MI)								
≤0.72	185/2020	7.4	1	<0.001	1	<0.001	1	0.01
>0.72 to ≤0.81	250/1996	10.4	1.41 (1.16–1.70)		1.29 (1.06–1.57)		1.22 (1.00, 1.50)	
>0.81 to ≤0.93	283/1981	11.6	1.64 (1.36–1.97)		1.31 (1.07–1.60)		1.18 (0.95–1.46)	
>0.93	382/1866	18.1	2.51 (2.11–2.99)		1.68 (1.36–2.09)		1.37 (1.07–1.74)	
CHD mortality								
≤0.72	71/2020	2.7	1	<0.001	1	<0.001	1	0.09
>0.72 to ≤0.81	118/1996	4.6	1.73 (1.29–2.32)		1.48 (1.09–2.00)		1.28 (0.94–1.74)	
>0.81 to ≤0.93	142/1981	6.1	2.14 (1.61–2.84)		1.48 (1.09–2.02)		1.12 (0.81–1.54)	
>0.93	234/1866	10.5	3.99 (3.06–5.21)		2.20 (1.60–3.03)		1.35 (0.95–1.93)	
Major CVD events (CVD mortality, nonfatal MI, stroke)								
≤0.72	222/2020	8.9	1	<0.001	1	<0.001	1	<0.001
>0.72 to ≤0.81	316/1996	13	1.50 (1.26–1.78)		1.34 (1.12–1.60)		1.29 (1.08–1.54)	
>0.81 to ≤0.93	353/1981	14.7	1.72 (1.45–2.03)		1.32 (1.10–1.58)		1.21 (1.00–1.47)	
>0.93	495/1866	22.8	2.75 (2.35–3.22)		1.77 (1.46–2.15)		1.47 (1.19–1.82)	
CVD mortality								
≤0.72	80/2020	3	1	<0.001	1	<0.001	1	0.03
>0.72 to ≤0.81	131/1996	5.1	1.70 (1.29–2.25)		1.42 (1.07–1.89)		1.25 (0.93–1.67)	
>0.81 to ≤0.93	164/1981	7.1	2.19 (1.68–2.86)		1.45 (1.09–1.94)		1.11 (0.82–1.51)	
>0.93	282/1866	12.3	4.27 (3.33–5.47)		2.27 (1.69–3.06)		1.44 (1.04–1.99)	
Stroke								
≤0.72	41/2020	1.8	1	<0.001	1	0.005	1	0.04
>0.72 to ≤0.81	72/1996	2.8	1.85 (1.26–2.71)		1.50 (1.01–2.23)		1.50 (1.01–2.24)	
>0.81 to ≤0.93	81/1981	3.3	2.13 (1.46–3.10)		1.44 (0.96–2.15)		1.42 (0.93–2.17)	
>0.93	116/1866	5.4	3.43 (2.40–4.90)		1.84 (1.20–2.81)		1.63 (1.02–2.59)	
Cancer mortality								
≤0.72	41/2020	1.4	1	<0.001	1	0.009	1	0.31
>0.72 to ≤0.81	55/1996	2.2	1.40 (0.93–2.10)		1.19 (0.78–1.80)		1.10 (0.72–1.68)	
>0.81 to ≤0.93	56/1981	2.2	1.47 (0.98–2.20)		1.15 (0.74–1.78)		0.93 (0.59–1.47)	
>0.93	81/1866	3.7	2.45 (1.68–3.56)		1.83 (1.16–2.88)		1.30 (0.78–2.14)	
Non-CVD non-cancer mortality								
≤0.72	15/2020	0.5	1	<0.001	1	0.42	1	0.95
>0.72 to ≤0.81	12/1996	0.6	0.85 (0.40–1.82)		0.71 (0.33–1.55)		0.68 (0.31–1.52)	
>0.81 to ≤0.93	20/1981	0.6	1.44 (0.74–2.82)		0.98 (0.47–2.03)		0.88 (0.40–1.94)	
>0.93	36/1866	1.5	2.86 (1.56–5.23)		1.37 (0.64–2.93)		0.97 (0.41–2.32)	

laboratory covariables, and other biomarkers including BNP and TnI. Not only baseline cystatin C levels, but also change in cystatin C from baseline to 1 year following randomization, had a significant association with most pre-specified cardiovascular outcomes at the end of the randomized trial period of 6 years,

Table 2. Continued

End point cystatin C mg/L	Events/total	5-y event rate (%)	Model 1, adjusted for only sex and treatment*		Model 2, adjusted for standard demographic and clinical risk factors†,‡		Model 3, adjusted for standard demographic, clinical risk factors, and all novel biomarkers‡,§	
			HR (95% CI)	*P* Value‖	HR (95% CI)	*P* Value‖	HR (95% CI)	*P* Value‖
All-cause mortality								
≤0.72	136/2020	4.9	1	<0.001	1	<0.001	1	0.02
>0.72 to ≤0.81	198/1996	7.7	1.52 (1.22–1.89)		1.27 (1.02–1.59)		1.14 (0.91–1.44)	
>0.81 to ≤0.93	240/1981	9.7	1.89 (1.53–2.34)		1.31 (1.04–1.64)		1.04 (0.81–1.32)	
>0.93	399/1866	16.8	3.57 (2.94–4.33)		2.03 (1.61–2.57)		1.35 (1.04–1.75)	

CHD indicates coronary heart disease; CVD, cardiovascular disease; HR, hazard ratio; and MI, myocardial infarction.

*These hazard ratios (HR) and 95% CI are adjusted for sex and treatment assignment only.

†These HRs and 95% CI are adjusted for baseline variables: age, sex, treatment assignment, stroke, diabetes, smoking, hypertension, total cholesterol, high-density lipoprotein cholesterol, nature of prior acute coronary syndrome, timing of coronary revascularization, systolic blood pressure, atrial fibrillation, creatinine estimated glomerular filtration rate, body mass index, dyspnea class, angina grade, white blood cell count, peripheral vascular disease, aspirin at baseline, proteinuria in spot urine.

‡These models have 47 (<1%) of patients removed because of at least 1 missing data value.

§These HR and 95% CI are adjusted for baseline variables: age, sex, treatment assignment, stroke, diabetes, smoking, hypertension, total cholesterol, high-density lipoprotein cholesterol, nature of prior acute coronary syndrome, timing of coronary revascularization, systolic blood pressure, atrial fibrillation, creatinine estimated glomerular filtration rate, body mass index, dyspnea class, angina grade, white blood cell count, peripheral vascular disease, aspirin at baseline, proteinuria in spot urine, brain natriuretic peptide, high-sensitivity C-reactive protein, D-dimer, lipoprotein(a), sensitive troponin I, mid-regional pro-adrenomedullin, and lipoprotein-associated phospholipase A2 activity.

‖This is the *P* value for trend for the biomarker.

and with cardiovascular, non-CVD non-cancer, and all-cause mortality after the total follow-up period of 16 years.

Consideration of BNP and TnI is particularly relevant, as among blood biomarkers, they have the most supportive evidence for the prediction of future CVD events.[28,29] Indeed, one of the earliest reports of the possible utility of a multiple biomarker score for cardiovascular event prediction showed that in elderly men with and without prevalent CVD, simultaneous addition of TnI, BNP, cystatin C, and hs-CRP substantially improved risk stratification for CVD mortality beyond a model based on conventional risk factors and confirmed the importance of cystatin C in this regard.[30] Here, we found that the predictive ability of cystatin C was probably inferior to BNP but similar to TnI. Although a sensitive rather than highly sensitive assay of TnI was used, this is unlikely to have affected our results.

It has previously been unclear how much of the prognostic value of cystatin C on CVD events and deaths relates to it being a better measure of eGFR or is attributable to other mechanisms. That a cystatin C-based definition of CKD is superior to a creatinine-based definition in assessing cardiovascular risk has been recently reported from analyses in 20 population-based and 3 disease cohorts.[31] Our study extends these observations by demonstrating that plasma cystatin C remains a significant predictor of major CVD events and long-term cause-specific as well as all-cause mortality after adjustment for eGFR using either the CKD-EPI creatinine or creatinine-cystatin C equations. Consequently, at least some of its prognostic value appears to be unrelated to it simply being a better measure of renal function. This contrasts with its prognostic value for development of CKD, which primarily related to it providing a better estimate of GFR, as discussed below.

Furthermore, eGFR, whether based on the CKD-EPI creatinine equation or the creatinine-cystatin C equation, was no longer associated with CVD end points over 6 years in the LIPID trial in models adjusted for only cystatin C quartiles, randomized treatment assignment and sex. Again, in contrast to cystatin C levels, eGFR-creatinine was only a weak predictor of long-term mortality outcomes.

The positive association between plasma cystatin C levels and cardiovascular outcomes and mortality has previously been demonstrated in large systematic reviews and meta-analyses. These have included subjects in the general population with normal eGFR,[32] those with CKD,[33] known or suspected CHD,[12] hypertension,[34] or heart failure.[35] Although some other studies have now incorporated long-term follow-up,[11,13,14] previously the duration of follow-up has typically been for much less than the 16-year data reported here.

Our data also show that in those patients with normal eGFR-creatinine at baseline, plasma cystatin C was also a significant predictor of development of renal dysfunction independent of baseline eGFR-creatinine and other CKD risk factors. Our data are

Table 3. Effect of Baseline Cystatin C Levels on Mortality Outcomes Over 16 Years

Outcome cystatin C level, mg/L	Events, n/N	15-y event rate, % (95% CI)	HR (95% CI)†	*P* Value
CHD mortality				
≤0.72	241/2020	11.9 (10.5–13.5)	1	<0.001
>0.72 to ≤0.81	358/1996	17.9 (16.2–19.8)	1.23 (1.04–1.45)	
>0.81 to ≤0.93	444/1981	23.8 (21.8–25.9)	1.29 (1.09–1.54)	
>0.93	601/1866	36.1 (33.6–38.6)	1.49 (1.24–1.79)	
CVD mortality				
≤0.72	297/2020	14.4 (12.9–16.1)	1	<0.001
>0.72 to ≤0.81	417/1996	20.2 (18.4–22.1)	1.14 (0.98–1.33)	
>0.81 to ≤0.93	542/1981	28.1 (26.0–30.3)	1.23 (1.05–1.43)	
>0.93	749/1866	42.7 (40.3–45.2)	1.42 (1.21–1.68)	
Cancer mortality				
≤0.72	147/2020	7.1 (6.0–8.4)	1	0.15
>0.72 to ≤0.81	202/1996	10.3 (8.9–11.8)	1.15 (0.92–1.43)	
>0.81 to ≤0.93	239/1981	13.4 (11.8–15.2)	1.22 (0.97–1.54)	
>0.93	242/1866	16.6 (14.6–18.8)	1.22 (0.93–1.59)	
Non-CVD non-cancer mortality				
≤0.72	102/2020	3.1 (2.4–4.1)	1	<0.001
>0.72 to ≤0.81	136/1996	4.8 (3.9–6.0)	1.17 (0.90–1.53)	
>0.81 to ≤0.93	184/1981	8.4 (7.1–10.0)	1.47 (1.13–1.91)	
>0.93	223/1866	13.8 (11.9–15.9)	1.86 (1.41–2.45)	
All-cause mortality				
≤0.72	546/2020	23.0 (21.2–24.9)	1	<0.001
>0.72 to ≤0.81	755/1996	31.8 (29.8–33.9)	1.16 (1.04–1.30)	
>0.81 to ≤0.93	965/1981	42.9 (40.8–45.2)	1.27 (1.13–1.44)	
>0.93	1214/1866	58.8 (56.5–61.1)	1.42 (1.25–1.63)	

CHD indicates coronary heart disease; CVD, cardiovascular disease; and HR, hazard ratio.

*Hazard ratios (HRs) and 95% CI were adjusted for trial treatment assignment and other baseline risk factors that remained significant after backward selection among age, sex, stroke, diabetes, smoking, hypertension, total cholesterol, high-density lipoprotein cholesterol, nature of prior acute coronary syndrome, timing of coronary revascularization, systolic blood pressure, atrial fibrillation, creatinine estimated glomerular filtration rate, body mass index, dyspnea class, angina grade, white blood cell count, peripheral vascular disease, triglyceride concentration, fasting glucose, aspirin at baseline, proteinuria in spot urine, brain natriuretic peptide, high-sensitivity C-reactive protein, D-dimer, lipoprotein(a), sensitive troponin I, mid-regional pro-adrenomedullin, and lipoprotein-associated phospholipase A2 activity.

†These models have 47 (<1%) of patients removed because of at least 1 missing data value.

again consistent with those of other investigators, who have also shown that plasma cystatin C can predict onset of CKD and its progression to end-stage renal disease.[33,36] However because this association was no longer significant with eGFR-creatinine-cystatin C in the statistical model, the prognostic value of circulating cystatin C appears to be related particularly to its superior estimation of renal function, with no significant effects unrelated to this mechanism.

Numerous cardiovascular biomarkers have been described.[37] In addition to their important role in the diagnosis of MI (cardiac troponins) and heart failure (BNP and NT-proBNP), biomarkers may have other applications. Our data confirm the possibility of refining risk stratification with estimation of cystatin C levels. However, the possible incorporation of biomarkers in usual practice also mandates other considerations. These include the availability and reliability of the assay and its cost-effectiveness. The assay for circulating cystatin C is not widely available. It is also expensive compared with the measurement of creatinine.[38] Although it is recommended in the United Kingdom for possible measurement in those with a creatinine-GFR 45, 59 mL/min per 1.73 m^2 and no albuminuria,[39] general use of eGFR-cystatin in the primary care setting in the United Kingdom has been shown to be not cost-effective.[40] Our data do not shed any light on the cost-effectiveness of measurement of cystatin C levels in the context of secondary prevention in patients with CHD. Also, although our findings demonstrate that changes in cystatin C levels were also related to outcomes not only in the medium term but extending to 16 years, this falls far short of the evidence needed to support use of serial

Table 4. Discrimination Results For Cystatin C, BNP, TnI, Age, and Qualifying Acute Coronary Syndrome Using Categorical NRI and C Statistics

	Categorical NRI	Net reclassification index				C Statistic	
Biomarker added to the base model	Prespecified risk cut-offs	NRI events	NRI nonevents	NRI	Bootstrap percentile CI	Base model*	Base model +biomarker*
RCT ≈6 y follow-up							
All-cause mortality	5%, 8%, 12%						
Cystatin C baseline		0.014	3.966	3.98	(1.50 to 10.76)	0.699	0.707
BNP baseline		–1.197	10.987	9.791	(–30.41 to 16.69)	0.699	0.719
TnI baseline		1.087	2.976	4.063	(–0.88 to 8.46)	0.699	0.707
Age, y		4.747	6.284	11.032	(1.08 to 8.21)	0.68	0.699
Qualifying ACS		–3.353	5.745	2.392	(5.71 to 16.05)	0.692	0.699
Coronary death	2.5%, 4%, 7%						
Cystatin C baseline		–0.691	3.667	2.976	(1.13 to 13.52)	0.727	0.734
BNP baseline		0.081	15.225	15.306	(7.83 to 22.99)	0.727	0.757
TnI baseline		4.662	5.74	10.402	(–1.14 to 11.46)	0.727	0.743
Age		–2.301	6.91	4.608	(4.10 to 16.17)	0.718	0.727
Qualifying ACS		–4.891	12.075	7.185	(0.40 to 14.76)	0.713	0.727
Coronary event (coronary death, nonfatal myocardial infarction)	7%, 10%, 14%						
Cystatin C baseline		2.032	2.081	4.113	(–0.51 to 9.54)	0.663	0.668
BNP baseline		–1.459	6.327	4.868	(1.89 to 12.31)	0.663	0.673
TnI baseline		3.395	3.87	7.264	(–0.60 to 9.33)	0.663	0.672
Age. y		1.046	1.993	3.039	(3.55 to 13.03)	0.658	0.663
Qualifying ACS		–4.664	11.335	6.671	(–1.44 to 9.43)	0.651	0.663
LTF ≈ 16 y follow-up							
All-cause mortality	22%, 35%, 52%						
Cystatin C baseline		1.427	1.82	3.247	(1.24 to 6.61)	0.696	0.701
BNP baseline		1.051	2.314	3.365	(1.74 to 7.63)	0.696	0.706
TnI baseline		–0.357	0.798	0.441	(0.32 to 5.22)	0.696	0.701
Age, y		6.911	7.563	14.473	(24.41 to 32.73)	0.651	0.696
Qualifying ACS		0.621	1.269	1.891	(0.71 to 5.12)	0.692	0.696
CVD death	13%, 21%, 35%						
Cystatin C baseline		0.138	1.391	1.529	(0.33 to 6.54)	0.713	0.717
BNP baseline		1.816	4.536	6.352	(7.13 to 14.64)	0.713	0.733
TnI baseline		0.923	2.11	3.033	(2.72 to 10.00)	0.713	0.724
Age, y		2.846	5.823	8.669	(14.88 to 24.30)	0.681	0.713
Qualifying ACS		0.16	2.466	2.626	(1.94 to 7.64)	0.703	0.713

CI derived using 2.5 and 97.5 percentiles from 1000 bootstraps. Base model includes: randomized treatment, stroke, diabetes, smoking, hypertension, total cholesterol, high-density lipoprotein cholesterol, age, sex, qualifying acute coronary syndrome, prior revascularization, systolic blood pressure, atrial fibrillation, estimated glomerular filtration rate, body mass index, dyspnea, angina, white blood cell count, peripheral vascular disease, aspirin use, and fasting glucose. ACS indicates acute coronary syndrome; BNP, brain natriuretic peptide; CVD, cardiovascular disease; LTF, long-term follow-up; NRI, net reclassification index; and TnI, troponin I.

*These models have 47 (<1%) patients removed because of at least 1 missing data value.

measurement of cystatin C to guide management of patients with CHD, as natriuretic peptides are in patients with heart failure.

Our confirmation of cystatin C as a cardiovascular risk marker does not shed further light on how it might impact on pathobiological processes. The pathogenesis of atherothrombotic disease associated with CKD is complex.[41] Risk is greater than explained by abnormalities in conventional risk factors,[42] and may relate to specific metabolic risk factors and mechanisms such as systemic inflammation, oxidative stress, and dyslipidemia.[41,42] Subclinical atherosclerosis develops in the

early stages of CKD and limited observational data have shown direct and independent associations of plasma cystatin C levels with carotid intima-media thickness.[43]

Our data are consistent with plasma cystatin C allowing a more sensitive assessment of "subclinical" renal damage than creatinine-based estimation of GFR,[38] extending into the "normal" GFR range as a continuous variable, in a manner similar to cholesterol and blood pressure. This may relate to several factors. The estimation of GFR in usual practice is adjusted for average body surface area, whereas adjustment for individual body surface area may overcome errors because of more extreme values. Creatinine, but not cystatin, is confounded by factors such as muscle mass. Also, the concept of the shrunken pore syndrome is consistent with larger molecules such as cystatin no longer passing through glomerular pores when smaller molecules such as creatinine are still able to do this.[44] Consequently, circulating levels of cystatin C would rise earlier.

Because the association of cystatin C levels with medium- and long-term outcomes might be greater than that expected from consideration of eGFR, it has been suggested that cystatin C might be implicated more directly in vascular disease.[10]

Cystatin C is an endogenous inhibitor of cathepsins, a family of cysteine proteases initially thought to be involved particularly in degradation of deleterious proteins in lysosomes.[45] It is now recognized that they are also involved in extracellular matrix protein degradation, cell signaling, cell migration, and apoptosis,[46–48] and have been implicated in the initiation and progression of atherosclerosis, and in plaque rupture. Human atherosclerotic plaques overexpress cathepsins but show deceased expression of cystatin C.[46,49,50]

Cystatin C and cathepsin expression in monocyte-derived macrophages from individual donors is highly variable and alters with disease states.[51] Differential regulation of tissue and plasma levels of cystatin C may explain the apparent discrepancy between the potential plaque stabilizing effects of tissue cystatin C and the deleterious associations of increased circulating cystatin C.

There has been considerable interest in the cardiorenal syndrome. A recent scientific statement from the American Heart Association describes the syndrome as encompassing "a spectrum of disorders involving both the heart and kidneys in which acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other organ".[52] Cystatin C is listed as one of the clinically relevant biomarkers in the syndrome. If cystatin C is a risk factor for CVD events (particularly heart failure, which was not examined in our study) this could be another mechanism for an increase in development of CKD. Conversely if cystatin C is a risk factor for developing CKD this might also be a mechanism for an increase in CVD events.

Finally, large Mendelian randomization studies and their meta-analysis appears to argue against cystatin C being causal and directly implicated in development of atherosclerosis. This is because genetic variants that increase cystatin C levels have not been associated with increased risk of cardiovascular events.[53,54] In contrast to our findings, these observations lend support to considering circulating cystatin C to be a powerful marker, rather than mediator of vascular risk, including in those with a "normal" eGFR.[55]

Strengths and Limitations

The strengths of this study include its prospective and comprehensive nature, with almost no missing data. The large cohort of stable patients with CHD were representative of those seen in usual practice, with detailed ascertainment and adjudication of all major CVD events during the trial period, and linkage to administrative data sets to establish cause-specific mortality during further follow-up to a total of 16 years. In addition, landmark analyses such as presented here are uncommonly performed, but are important and may account for regression to the mean with repeated measures. Although the landmark analyses do not provide information about the highest risk patients who had a fatal event within the first 12 months after randomization, such events were captured in analyses involving the baseline assays.

In risk-adjusted models describing cardiovascular, renal, and mortality outcomes we adjusted for proteinuria in a single spot urine test at baseline, but did not formally measure urinary albumin-creatinine ratio, another important renal marker that predicts cardiovascular outcomes[38,56] and mortality[38,56] independently of eGFR and cystatin C. Albuminuria may reflect not only glomerular damage, but damage and endothelial dysfunction in other vascular beds.[57]

The initial 6-year randomized double-masked LIPID study was performed >20 years ago. However, most patients consented to long-term follow-up, allowing our observations to extend to 16 years following randomization. Although patients in the study were otherwise well treated according to contemporary evidence, there have since been some changes in acute and chronic cardiovascular management. New diagnostic criteria for acute MI are now also in place. However, the impact of these is minimized as participants in the LIPID study were randomized at a median time of 1 year following their qualifying event. Because of this, they can be considered to have stable CHD rather than representing a cohort randomized early after an acute coronary syndrome.

While methods such as cross-validation are valuable for the development of comprehensive predictive models this is beyond the scope of the current paper which has the primary purpose of exploring cystatin C as a useful risk factor, its relevance for pathobiology

and assessing its importance for possible inclusion in future risk models.

CONCLUSIONS

The association of elevated circulating levels of cystatin C with adverse medium- and long-term outcomes underscores the importance of CKD as a cardiovascular risk factor in our aging societies. However, our data also support the additional prognostic value for major CVD events and long-term mortality of measurement of circulating cystatin C beyond allowing improved assessment of eGFR.

APPENDIX

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Supplemental Material

Tables S1–S7
Figures S1–S3

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SUPPLEMENTAL MATERIAL

Table S1: Additional biomarkers analyzed and their characteristics

Process	Marker	Assay range	Coefficient of variation
Myocardial stress	BNP	<5000 pg/mL	5.2%
Myocardial injury	Tn I	0.006–50 ng/mL	10% (at 0.03 ng/mL)
Inflammation	hs-CRP	0.01–160 mg/dL	3.8%
Plaque instability	Lp-PLA2	3.62–724 nmol/min/mL	3.8%
Neurohumoral	Midregional pro-adrenomedullin	0.05–10 nmol/L	20% (at 0.25 nmol/L)
Lipids	Lp(a)	1.3–90 mg/dL	4.6%
Coagulation	D-dimer	0–1600 ng/mL	6.4%

Table S2: Association of Baseline Cystatin C Levels and Risk of 6-Year Cardiovascular Endpoints, Adjusted for Risk Factors including i) eGFR-creatinine and ii) eGFR-cystatin C-creatinine

Cystatin C (mg/L)	Model adjusted for eGFR-creatinine†		Model adjusted for eGFR-cystatin C-creatinine†	
	HR (95% CI)	P-value**	HR (95% CI)	P-value**
CHD events (CHD mortality and non-fatal MI)				
≤0.72	1	0.01	1	0.08
>0.72–≤0.81	1.22 (1.00, 1.50)		1.22 (0.99, 1.51)	
>0.81–≤0.93	1.18 (0.95, 1.46)		1.18 (0.92, 1.51)	
>0.93	1.37 (1.07, 1.74)		1.31 (0.97, 1.77)	
CHD mortality				
≤0.72	1	0.09	1	0.12
>0.72–≤0.81	1.28 (0.94, 1.74)		1.33 (0.96, 1.84)	
>0.81–≤0.93	1.12 (0.81, 1.54)		1.19 (0.82, 1.73)	
>0.93	1.35 (0.95, 1.93)		1.41 (0.91, 2.19)	
Major CVD events (CVD mortality, non-fatal MI, stroke)				
≤0.72	1	<.001	1	0.02
>0.72–≤0.81	1.29 (1.08, 1.54)		1.27 (1.05, 1.54)	
>0.81–≤0.93	1.21 (1.00, 1.47)		1.19 (0.95, 1.49)	
>0.93	1.47 (1.19, 1.82)		1.37 (1.05, 1.79)	
CVD mortality				
≤0.72	1	0.03	1	0.15
>0.72–≤0.81	1.25 (0.93, 1.67)		1.27 (0.93, 1.73)	
>0.81–≤0.93	1.11 (0.82, 1.51)		1.13 (0.80, 1.60)	
>0.93	1.44 (1.04, 1.99)		1.35 (0.90, 2.04)	

**This is the p value for trend for the biomarker.

^These HR and 95% CI are adjusted for baseline variables: age, sex, treatment assignment, stroke, diabetes, smoking, hypertension, total cholesterol, HDL-c, nature of qualifying ACS, timing of coronary revascularization, SBP, atrial fibrillation, creatinine eGFR or cystatin C-creatinine eGFR, BSA, BMI, dyspnea class, angina grade, WBC count, peripheral vascular disease, aspirin use at baseline, proteinuria in spot urine, brain natriuretic peptide, hs-CRP, D-dimer, lipoprotein(a), sensitive troponin I, mid-regional pro-adrenomedullin, and lipoprotein-associated phospholipase A2 activity.

†These models have 47 (<1%) of patients removed because of at least one missing data value.

CHD = Coronary heart disease, CVD = Cardiovascular disease, MI=Myocardial infarction; HR = hazard ratio; CI = confidence interval.

Table S3: Effect of Pravastatin on Cardiovascular Events During the LIPID Study for each Quartile of Cystatin C

Endpoint	Cystatin C level, mg/L	Placebo % events	Pravastatin % events	HR (95% CI)	NNT (Common hazard)	Interaction P trend†
CHD events (CHD mortality and non-fatal MI)						
	≤0.72	10.0	8.3	0.82 (0.61, 1.10)	62	0.62
	>0.72–≤0.81	14.1	11.0	0.77 (0.60, 0.98)	42	
	>0.81–≤0.93	16.6	12.1	0.70 (0.55, 0.89)	38	
	>0.93	21.9	19.0	0.86 (0.70, 1.05)	28	
Major CVD events (CVD mortality, non-fatal MI, stroke)						
	≤0.72	12.0	10.0	0.83 (0.64, 1.08)	50	0.77
	>0.72–≤0.81	17.5	14.2	0.80 (0.64, 0.99)	34	
	>0.81–≤0.93	21.2	14.6	0.66 (0.54, 0.82)	29	
	>0.93	28.5	24.5	0.84 (0.70, 1.00)	21	
Stroke						
	≤0.72	2.0	2.1	1.07 (0.58, 1.97)	248	0.85
	>0.72–≤0.81	4.2	3.0	0.70 (0.44, 1.12)	125	
	>0.81–≤0.93	5.0	3.2	0.63 (0.40, 0.98)	104	
	>0.93	6.8	5.6	0.81 (0.56, 1.17)	68	
CHD mortality						
	≤0.72	4.0	3.0	0.74 (0.46, 1.18)	145	0.13
	>0.72–≤0.81	7.2	4.7	0.64 (0.44, 0.92)	79	
	>0.81–≤0.93	8.4	6.0	0.70 (0.50, 0.97)	65	
	>0.93	13.0	12.1	0.92 (0.71, 1.19)	46	
CVD mortality						
	≤0.72	4.6	3.3	0.71 (0.46, 1.11)	122	0.17
	>0.72–≤0.81	7.7	5.5	0.70 (0.49, 0.98)	72	
	>0.81–≤0.93	9.8	6.8	0.67 (0.49, 0.92)	54	
	>0.93	15.9	14.3	0.89 (0.70, 1.12)	38	
Cancer mortality						
	≤0.72	2.0	2.1	1.06 (0.58, 1.96)	514	0.87
	>0.72–≤0.81	3.0	2.5	0.83 (0.49, 1.41)	280	
	>0.81–≤0.93	3.6	2.1	0.56 (0.33, 0.96)	259	
	>0.93	4.3	4.4	1.01 (0.65, 1.56)	204	
Non-CVD non-cancer mortality						
	≤0.72	1.0	0.5	0.50 (0.17, 1.48)	354	0.59
	>0.72–≤0.81	0.8	0.4	0.46 (0.14, 1.54)	310	
	>0.81–≤0.93	1.2	0.8	0.62 (0.25, 1.52)	413	
	>0.93	2.3	1.5	0.67 (0.34, 1.32)	166	
All-cause mortality						
	≤0.72	7.5	5.9	0.77 (0.55, 1.09)	82	0.2
	>0.72–≤0.81	11.5	8.4	0.71 (0.54, 0.95)	50	
	>0.81–≤0.93	14.6	9.7	0.64 (0.50, 0.83)	41	
	>0.93	22.5	20.2	0.89 (0.73, 1.08)	29	

*These HR and 95% CI are from the model with no interaction term, adjusted for sex only.

† Test for interaction not significant for any category.

CHD = Coronary heart disease, CVD = Cardiovascular disease, MI=Myocardial infarction; HR = hazard ratio; CI = confidence interval; NNT = number needed to treat.

Table S4: Association of Baseline Cystatin C Levels and Mortality Outcomes Over 16 Years: Adjusted for Risk Factors Including i) eGFR-creatinine ii) eGFR-cystatin C-creatinine

	Model adjusted for eGFR-creatinine		Model adjusted for eGFR-cystatin C-creatinine	
Outcome Cystatin C Level, mg/L	HR (95% CI)[†]	P-value	HR (95% CI)[†]	P-value
Coronary death				
≤0.72	1	<.001	1	.002
>0.72–≤0.81	1.23 (1.04, 1.45)		1.26 (1.06, 1.51)	
>0.81–≤0.93	1.29 (1.09, 1.54)		1.34 (1.09, 1.64)	
>0.93	1.49 (1.24, 1.79)		1.45 (1.14, 1.84)	
Cardiovascular disease death (CVD)				
≤0.72	1	<.001	1	0.10
>0.72–≤0.81	1.14 (0.98, 1.33)		1.16 (0.99, 1.37)	
>0.81–≤0.93	1.23 (1.05, 1.43)		1.24 (1.03, 1.49)	
>0.93	1.42 (1.21, 1.68)		1.31 (1.05, 1.63)	
Cancer death				
≤0.72	1	0.15	1	0.37
>0.72–≤0.81	1.15 (0.92, 1.43)		1.11 (0.88, 1.40)	
>0.81–≤0.93	1.22 (0.97, 1.54)		1.15 (0.88, 1.51)	
>0.93	1.22 (0.93, 1.59)		1.16 (0.83, 1.63)	
Non-CVD non-cancer death				
≤0.72	1.17 (0.90, 1.53)	<.001	1	<.001
>0.72–≤0.81	1.47 (1.13, 1.91)		1.22 (0.92, 1.61)	
>0.81–≤0.93	1.86 (1.41, 2.45)		1.72 (1.26, 2.33)	
>0.93	1.91 (1.45, 2.51)		2.37 (1.64, 3.41)	
All-cause mortality				
≤0.72	1	<.001	1	<.001
>0.72–≤0.81	1.16 (1.04, 1.30)		1.17 (1.03, 1.32)	
>0.81–≤0.93	1.27 (1.13, 1.44)		1.30 (1.13, 1.49)	
>0.93	1.42 (1.25, 1.63)		1.41 (1.19, 1.67)	

*HR and 95% CI were adjusted for trial treatment assignment and other baseline risk factors that remained significant after backward selection among age, sex, stroke, diabetes mellitus, smoking, hypertension, total cholesterol, high-density lipoprotein cholesterol, nature of prior acute coronary syndrome, timing of coronary revascularization, systolic blood pressure, atrial fibrillation, creatinine eGFR or cystatinC/creatinine eGFR, BSA, BMI, dyspnea class, angina grade, white blood cell count, peripheral vascular disease, triglyceride concentration, fasting glucose, aspirin at baseline, proteinuria in spot urine, brain natriuretic peptide, high-sensitivity C-reactive protein, D-dimer, lipoprotein(a), sensitive troponin I, mid-regional pro-adrenomedullin, and lipoprotein-associated phospholipase A2 activity.

†These models have 47 (<1%) of patients removed because of at least one missing data value.

CHD= Coronary heart disease, CVD = cardiovascular disease, HR= hazard ratio, CI= confidence interval.

Table S5. Associations Between Baseline Cystatin C Levels and Cause-specific and All-cause Mortality Within 5-Year Periods, After Adjustment for Other Baseline Risk Factors*†

Time period		0 to 5 years		5 to 10 years		10 to 15 years	
Outcome		HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
CHD mortality	≤0.72	1	0.002	1	0.001	1	<0.001
	>0.72–≤0.81	1.41 (1.00, 1.98)		1.09 (0.81, 1.45)		1.46 (1.14, 1.88)	
	>0.81–≤0.93	1.49 (1.07, 2.08)		1.21 (0.92, 1.61)		1.65 (1.28, 2.12)	
	>0.93	1.91 (1.38, 2.63)		1.58 (1.20, 2.09)		2.11 (1.64, 2.72)	
CVD mortality	≤0.72	1	<0.001	1	<0.001	1	<0.001
	>0.72–≤0.81	1.35 (0.98, 1.86)		1.04 (0.80, 1.36)		1.32 (1.05, 1.65)	
	>0.81–≤0.93	1.45 (1.07, 1.97)		1.13 (0.87, 1.45)		1.58 (1.27, 1.97)	
	>0.93	1.88 (1.40, 2.53)		1.56 (1.22, 2.01)		1.97 (1.57, 2.46)	
Cancer mortality	≤0.72	1	0.045	1	0.38	1	0.05
	>0.72–≤0.81	1.23 (0.76, 1.99)		0.98 (0.68, 1.41)		1.37 (1.00, 1.90)	
	>0.81–≤0.93	1.06 (0.65, 1.73)		1.26 (0.89, 1.79)		1.63 (1.18, 2.24)	
	>0.93	1.54 (0.96, 2.45)		1.28 (0.88, 1.85)		1.75 (1.24, 2.47)	
Non-CVD non-cancer mortality	≤0.72	1	0.26	1	0.003	1	<0.001
	>0.72–≤0.81	0.90 (0.38, 2.13)		0.87 (0.45, 1.68)		1.37 (1.01, 1.86)	
	>0.81–≤0.93	0.73 (0.31, 1.74)		1.82 (1.04, 3.18)		1.65 (1.21, 2.23)	
	> 0.93	1.41 (0.66, 2.98)		2.59 (1.50, 4.48)		2.06 (1.49, 2.84)	
All-cause mortality	≤ 0.72	1	<0.001	1	<0.001	1	<0.001
	>0.72–≤0.81	1.29 (1.00, 1.66)		1.00 (0.82, 1.23)		1.34 (1.15, 1.57)	
	>0.81–≤0.93	1.29 (1.01, 1.65)		1.23 (1.01, 1.49)		1.60 (1.37, 1.87)	
	>0.93	1.81 (1.43, 2.29)		1.57 (1.29, 1.90)		1.92 (1.63, 2.25)	

* HR and 95% CI are adjusted for baseline cystatin C level, treatment assignment, and other significant baseline risk factors among age, sex, stroke, diabetes, smoking, hypertension, total cholesterol, Apo B, Apo A1, HDL-c, nature of prior ACS, timing of coronary revascularization, SBP, atrial fibrillation, eGFR, BMI, dyspnea class, angina grade, WBC, peripheral vascular disease, triglycerides, fasting glucose, proteinuria in spot urine and aspirin treatment. CHD = coronary heart disease; CVD = cardiovascular disease; HR = hazard ratio; CI= confidence interval.

†These models have 47 (<1%) of patients removed because of at least one missing data value.

Table S6: Effects of Change to One Year in Cystatin C Levels on Risk of Outcomes During the LIPID Study

Cystatin C change (mg/L)	Events/ Total	5-Year event rate (95% CI)	HR (95% CI)†	P value trend
CHD event (CHD mortality and non-fatal MI)				
≤ -0.05	207/1720	10.0 (8.6, 11.5)	1	0.002
>-0.05 –≤0	223/1957	9.4 (8.2, 10.8)	1.07 (0.88, 1.30)	
>0 – ≤0.04	171/1529	9.0 (7.6, 10.5)	1.11 (0.90, 1.37)	
>0.04	244/1621	12.7 (11.1, 14.4)	1.35 (1.12, 1.64)	
Major CVD Event (CVD mortality, non-fatal MI, any stroke)				
≤ -0.05	267/1720	12.7 (11.2, 14.4)	1	<.001
>-0.05 – ≤0	279/1957	12.1 (10.8, 13.7)	1.06 (0.89, 1.26)	
>0 – ≤0.04	222/1529	11.7 (10.1, 13.4)	1.13 (0.94, 1.36)	
> 0.04	319/1621	16.4 (14.6, 18.3)	1.37 (1.15, 1.62)	
Any stroke				
≤ -0.05	62/1720	3.0 (2.3, 3.9)	1	0.030
>-0.05 – ≤0	69/1957	3.1 (2.4, 4.0)	1.17 (0.82, 1.66)	
>0 – ≤0.04	54/1529	2.8 (2.1, 3.8)	1.21 (0.83, 1.77)	
> 0.04	83/1621	4.1 (3.2, 5.2)	1.43 (1.02, 2.01)	
CHD mortality				
≤ -0.05	104/1720	4.9 (4.0, 6.1)	1	0.24
>-0.05 – ≤0	97/1957	3.7 (3.0, 4.7)	0.95 (0.71, 1.26)	
>0 – ≤0.04	79/1529	3.8 (2.9, 4.9)	1.10 (0.81, 1.49)	
> 0.04	114/1621	5.6 (4.6, 6.8)	1.19 (0.90, 1.56)	
CVD mortality				
≤ -0.05	434/1719	5.6 (4.6, 6.8)	1	0.051
>-0.05 – ≤0	416/1957	4.3 (3.5, 5.3)	0.96 (0.74, 1.26)	
>0 – ≤0.04	330/1529	4.5 (3.6, 5.7)	1.15 (0.87, 1.52)	
> 0.04	483/1620	6.8 (5.6, 8.1)	1.29 (1.00, 1.66)	
Cancer mortality				
≤ -0.05	184/1719	2.0 (1.4, 2.8)	1	0.08
>-0.05 – ≤0	199/1957	1.6 (1.1, 2.3)	0.94 (0.62, 1.44)	
>0 – ≤0.04	150/1529	2.0 (1.4, 2.9)	1.04 (0.67, 1.63)	
> 0.04	184/1620	2.9 (2.2, 3.9)	1.41 (0.95, 2.08)	
Non-CVD non-cancer mortality				
≤ -0.05	132/1719	0.6 (0.3, 1.1)	1	0.85
>-0.05 – ≤0	150/1957	0.4 (0.2, 0.8)	0.74 (0.34, 1.58)	
>0 – ≤0.04	128/1529	0.9 (0.5, 1.5)	1.34 (0.65, 2.76)	
> 0.04	156/1620	0.8 (0.4, 1.4)	0.94 (0.47, 1.90)	
All-cause mortality				
≤ -0.05	750/1719	8.0 (6.8, 9.4)	1	0.019
>-0.05 – ≤0	765/1957	6.2 (5.2, 7.3)	0.93 (0.75, 1.15)	
>0 – ≤0.04	608/1529	7.3 (6.1, 8.7)	1.12 (0.89, 1.40)	
>0.04	823/1620	10.2 (8.8, 11.8)	1.27 (1.04, 1.55)	

**This is the p value for trend for the biomarker change quartiles.

*These HR and 95% CI are from the model with no interaction term, adjusted for baseline variables: age, sex, treatment assignment , stroke, diabetes, smoking, hypertension, total cholesterol, Apo B, Apo A1, HDL-c, Nature of prior ACS, coronary revascularization, SBP, atrial fibrillation, eGFR, BMI, dyspnea class, angina grade, WBC, peripheral vascular disease, aspirin at baseline, proteinuria, BNP, hs-CRP, D-dimer, Lp(a), sens TNI, MR-pro-adrenomedullin and lipoprotein-associated phospholipase A2 activity.

CHD = coronary heart disease, CVD = cardiovascular disease, MI = myocardial infarction, HR = hazard ratio, CI = confidence interval.

†These models have 47 (<1%) of patients removed because of at least one missing data value.

Table S7: Effect of Changes in Cystatin C Levels from Baseline to 12 Months on Cause-specific and All-cause Mortality Over 15 Years' Follow-up, After Adjustment for Baseline Cystatin C and Other Risk Factors*

Endpoint	Cystatin C change (mg/mL)	Events/total	14 year event rate after Year 1	HR (95% CI)*†	P trend*
CHD mortality					
Change to Year 1	≤ -0.05	356/1719	22.0 (20.0, 24.2)	1	0.004
	>-0.05 – ≤0	350/1957	17.7 (16.0, 19.6)	0.97 (0.84, 1.13)	
	>0 – ≤0.04	267/1529	17.7 (5.7, 19.8)	1.03 (0.87, 1.22)	
	> 0.04	380/1620	25.3 (23.1, 27.7)	1.26 (1.08, 1.46)	
CVD mortality					
Change to Year 1	≤ -0.05	434/1719	26.0 (23.9, 28.3)	1	<0.001
	>-0.05 – ≤0	416/1957	20.8 (19.0, 22.8)	0.96 (0.84, 1.11)	
	>0 – ≤0.04	330/1529	20.9 (18.8, 23.1)	1.06 (0.91, 1.23)	
	> 0.04	483/1620	30.4 (28.1, 32.9)	1.31 (1.15, 1.50)	
Cancer mortality					
Change to Year 1	≤ -0.05	184/1719	11.8 (10.2, 13.6)	1	0.23
	>-0.05 – ≤0	199/1957	10.5 (9.1, 12.1)	0.98 (0.80, 1.20)	
	>0 – ≤0.04	150/1529	10.5 (9.0, 12.4)	0.99 (0.79, 1.24)	
	> 0.04	184/1620	12.4 (10.7, 14.3)	1.11 (0.90, 1.37)	
Non-CVD non-cancer mortality					
Change to Year 1	≤ -0.05	132/1719	6.7 (5.5, 8.2)	1	0.002
	>-0.05 – ≤0	150/1957	6.3 (5.2, 7.6)	1.11 (0.87, 1.41)	
	>0 – ≤0.04	128/1529	6.4 (5.2. 8.0)	1.27 (0.99, 1.64)	
	> 0.04	156/1620	7.9 (6.5, 9.6)	1.43 (1.13, 1.83)	
All-cause mortality					
Change to Year 1	≤ -0.05	750/1719	39.2 (36.9, 41.6)	1	<0.001
	>-0.05 – ≤0	765/1957	33.6 (31.5, 35.8)	0.99 (0.89, 1.10)	
	>0 – ≤0.04	608/1529	33.8 (31.4, 36.2)	1.07 (0.96, 1.20)	
	> 0.04	823/1620	43.8 (41.4, 46.3)	1.27 (1.15, 1.41)	

*HR and 95% CI are adjusted for baseline cystatin C concentration, treatment assignment, and other significant risk factors from age, sex, stroke, diabetes, smoking, hypertension, total cholesterol, Apo B, Apo A1, HDL-c, nature of qualifying prior ACS, timing of coronary revascularization, SBP, atrial fibrillation, eGFR, BMI, dyspnea class, angina grade, WBC, peripheral vascular disease, triglycerides, fasting glucose, proteinuria, aspirin assignment at baseline, BNP, hs-CRP, D-dimer, Lp(a), sens itiveTnI, MR-pro-adrenomedullin and lipoprotein-associated phospholipase A2 activity.
CHD = coronary heart disease, CVD = cardiovascular disease, HR = hazard ratio, CI = confidence interval.
†These models have 47 (<1%) of patients removed because of at least one missing data value.

Figure S1: Consort Diagram

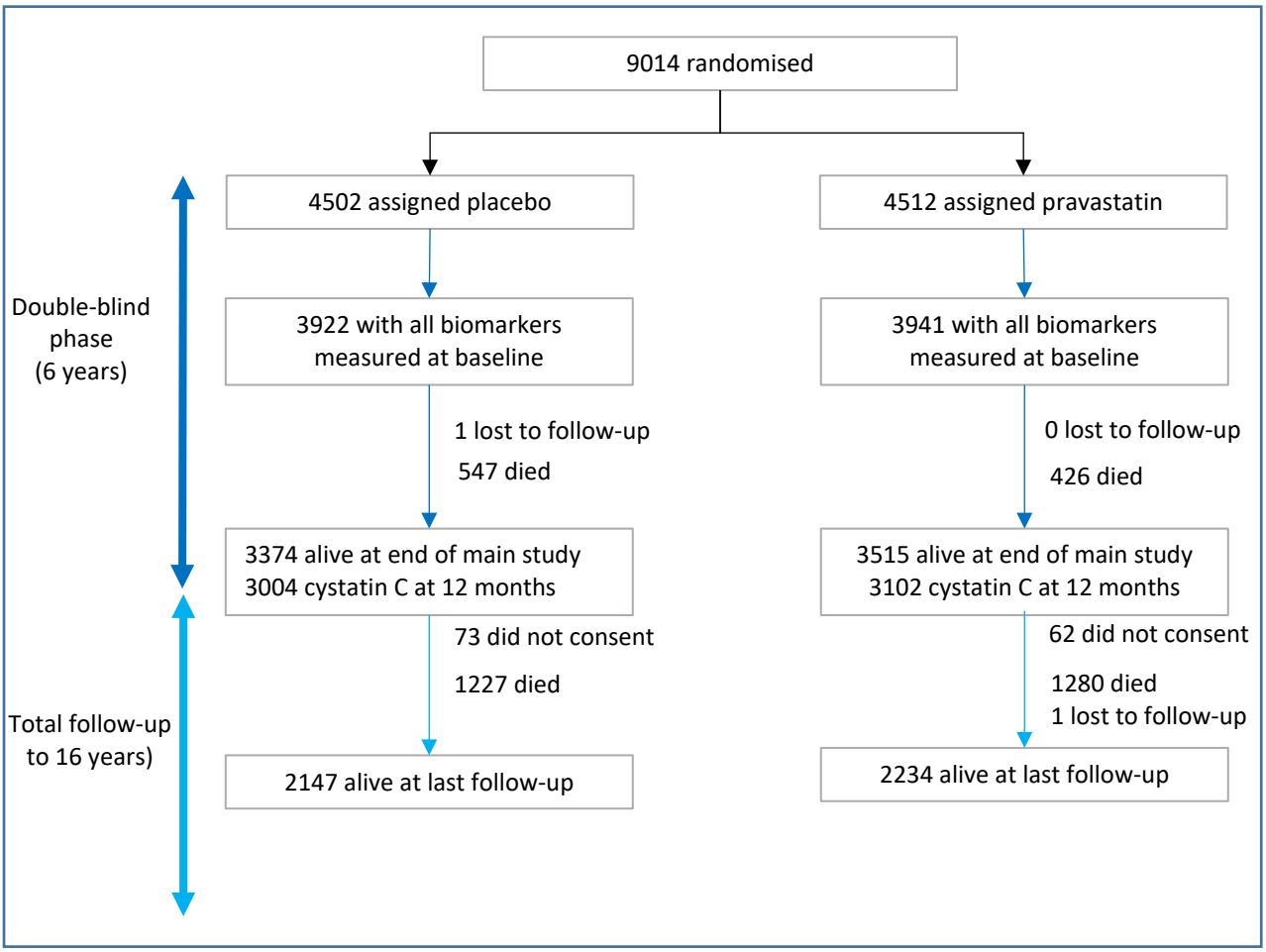
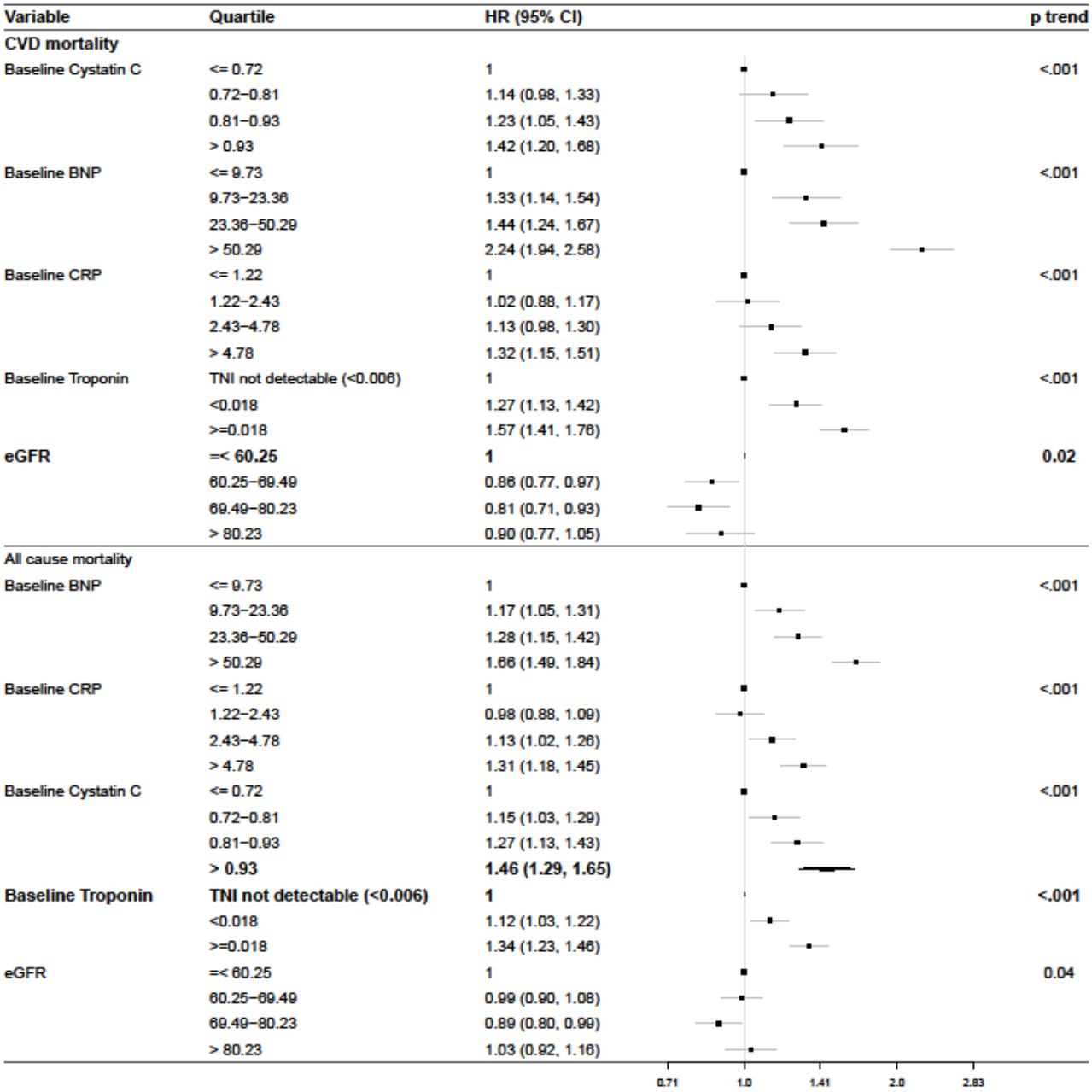
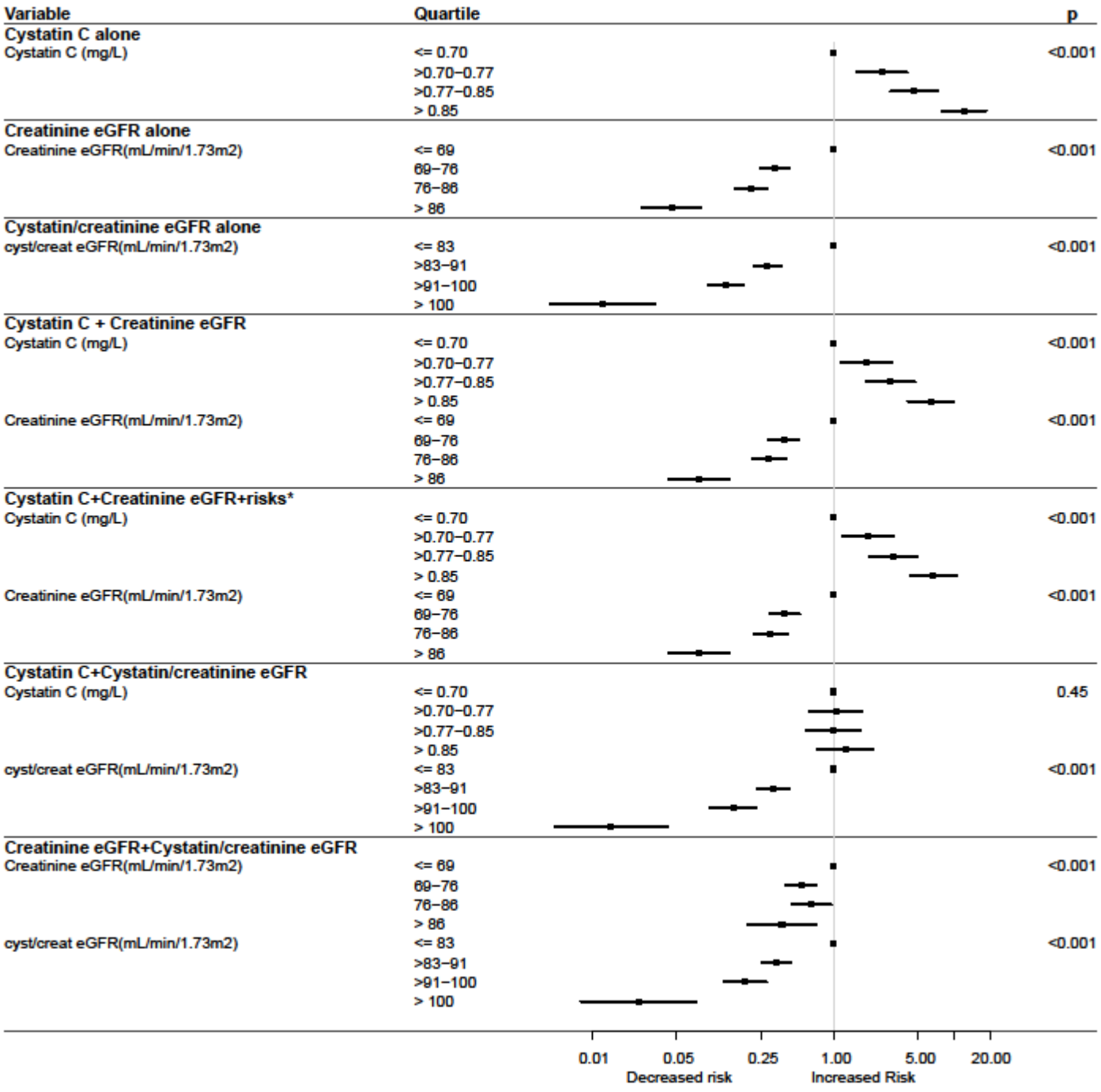


Figure S2: Forest Plot Showing Relationships of Different Biomarkers to Cardiovascular and All-cause Mortality over 16 years' Follow-up†



CVD = cardiovascular disease, BNP = brain natriuretic peptide, eGFR = estimated glomerular filtration rate, HR= hazard ratio, CI = confidence intervals.
*HR and 95% CI were, adjusted for trial treatment assignment and other risk factors that remained significant after backward selection: among age, sex, stroke, diabetes mellitus, smoking, hypertension, total cholesterol, high-density lipoprotein cholesterol, nature of prior acute coronary syndrome, timing of coronary revascularization, systolic blood pressure, atrial fibrillation, estimated glomerular filtration rate (eGFR) , body mass index, dyspnea class, angina grade, white blood cell count, peripheral vascular disease, triglyceride concentration, fasting glucose, aspirin at baseline, proteinuria in spot urine, brain natriuretic peptide, hs-CRP, D-dimer, lipoprotein(a), sensitive troponin I, mid-regional pro-adrenomedullin, and lipoprotein-associated phospholipase A2 activity.
†These models have 47 (<1%) of patients removed because of at least one missing data value.

Figure S3: Associations of Circulating Cystatin C and Different Measures of eGFR with the Development of Chronic Kidney Disease†



*Adjusted for eGFR, sex, diabetes mellitus, hypertension, body surface area, HDL-cholesterol, triglycerides and proteinuria at baseline.
†These models have 47 (<1%) of patients removed because of at least one missing data value.