ORIGINAL RESEARCH

Biomarker of Collagen Turnover (C-Terminal Telopeptide) and Prognosis in Patients With Non-ST-Elevation Acute Coronary Syndromes

Thomas A. Zelniker, MD, MSc; Petr Jarolim, MD, PhD; Benjamin M. Scirica, MD, MPH; Eugene Braunwald, MD; Jeong-Gun Park, PhD; Saumya Das, MD, PhD; Marc S. Sabatine, MD, MPH; David A. Morrow, MD, MPH

***Background*—**Small studies have suggested an association between markers of collagen turnover and adverse outcomes in heart failure (HF). We examined C-terminal telopeptide (beta-CTx) and the risk of cardiovascular death or new or worsening HF in non–ST-elevation acute coronary syndrome.

***Methods and Results*—**We measured baseline serum beta-CTx, NT-proBNP (N-terminal pro-B-type natriuretic peptide), hsTnT (high-sensitivity cardiac troponin T) and hsCRP (high-sensitivity C-reactive protein) (Roche Diagnostics) in a nested biomarker analysis (n=4094) from a study of patients with non–ST-elevation acute coronary syndrome. The relationship between quartiles of beta-CTx and cardiovascular death or HF over a median follow-up time of 12 months was analyzed using adjusted Cox models. Higher beta-CTx levels identified a significantly higher risk of cardiovascular death/HF (Q4 10.9% versus Q1 3.8%, Logrank $P<0.001$). After multivariable adjustment, beta-CTx in the top quartile (Q4) was associated with cardiovascular death/HF (Q4 versus Q1: adjusted hazard ratio 2.22 [1.50–3.27]) and its components (Q4 versus Q1: cardiovascular death: adjusted hazard ratio 2.48 [1.46–4.21]; HF: adjusted hazard ratio 2.04 [1.26–3.30]). In an adjusted multimarker model including NT-proBNP, hsTnT, and hsCRP, beta-CTx remained independently associated with cardiovascular death/HF (Q4 versus Q1: adjusted hazard ratio 1.98 [1.34–2.93]) and its components. Beta-CTx correlated weakly with NT-proBNP ($r=0.17$, $P<0.001$) and left ventricular ejection fraction ($r=-0.05$, $P=0.008$) and did not correlate with hsTnT ($r=0.02$, $P=0.20$), or hsCRP ($r=-0.03$, $P=0.09$).

***Conclusions*—**Levels of beta-CTx, a biomarker of collagen turnover, were associated with cardiovascular death and HF in patients with non–ST-elevation acute coronary syndrome. This biomarker, which correlated only weakly or not significantly with traditional biomarkers of cardiovascular death and HF, may provide complementary pathobiological insight and risk stratification in these patients. **(*J Am Heart Assoc*. 2019;8:e011444. DOI: 10.1161/JAHA.118.011444.)**

Key Words: beta-CTx • cardiovascular death • collagen turnover • C-terminal telopeptide • heart failure

The predominance of adverse outcomes in patients with ischemic heart disease is shifting from recurrent ischemic events to complications related to myocardial dysfunction and heart failure (HF). Myocardial stress and injury can trigger heterogenous mechanisms that activate myofibroblasts and result in excessive collagen deposition in the extracellular matrix.[1] Accumulation of collagen is involved in the progression of myocardial fibrosis, which is a central pathobiological substrate in adverse remodeling, development of HF and sudden cardiac death in patients with ischemic heart disease.[1–5] Although the properties of myocardial collagen are not completely understood, imbalances between the synthesis and degradation, as well as in the ratio of the collagen subtypes modify the biomechanical properties of the myocardium.[6,7] In addition, excessive cross-linking can alter the quantity (by impeding its degradation) and quality of collagen.[8–10] As such, an increase in collagen Type I fibers has been associated with cardiac stiffness and observed in patients with hypertensive heart disease and aortic stenosis.[4,8]

C-terminal telopeptide (beta-CTx) is a fragment of Type I collagen that is released into the bloodstream during its degradation. As a biomarker of collagen turnover, it is of

From the TIMI Study Group, Division of Cardiovascular Medicine (T.A.Z., B.M.S., E.B., J.-G.P., M.S.S., D.A.M.) and Department of Pathology (P.J.), Brigham and Women's Hospital and Harvard Medical School, Boston, MA; Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School Boston, MA (S.D.).

Accompanying Data S1, Tables S1, S2 and Figures S1 through S6 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.011444

Correspondence to: David A. Morrow, MD, MPH, TIMI Study Group, 60 Fenwood Rd., Suite 7022-7024W, Boston, MA 02115. E-mail: dmorrow@bwh.harvard.edu

Received November 29, 2018; accepted March 25, 2019.

© 2019 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Clinical Perspective

What Is New?

- C-terminal telopeptide is a marker of collagen turnover and therefore a potential candidate biomarker for early detection of adverse remodeling.
- Levels of C-terminal telopeptide were associated with cardiovascular death and heart failure and its individual components in patients with non–ST-elevation acute coronary syndrome.

What Are the Clinical Implications?

- C-terminal telopeptide, which correlated only weakly or not significantly with traditional biomarkers of cardiovascular death and heart failure, may provide complementary pathobiological insight in these patients.
- These data lend support to investigate collagen metabolism as a potential therapeutic target.

interest as a potential candidate biomarker to assist in early detection of disease, provide prognostic information, and/or add pathophysiological insights.[3,11] Therefore, we aimed to examine the association of beta-CTx with incident HF and cardiovascular death, including sudden cardiac death, in a large cohort of patients with non–ST-elevation acute coronary syndrome.

Materials and Methods

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. However, we encourage parties interested in collaboration and data sharing to contact the corresponding author directly for further discussions.

Patient Population

The design and primary results of the MERLIN (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST Elevation Acute Coronary Syndromes)-TIMI 36 trial have been published previously.[12,13] In brief, patients eligible for enrollment had at least 10 minutes of ischemic symptoms at rest and presented with one of the following additional risk indicators: elevated biomarkers of myonecrosis, ST depression ≥0.1 mV, history of diabetes mellitus, or an intermediate to high (≥3) TIMI risk score. Patients were excluded if they had clinically significant liver disease, end-stage renal disease requiring dialysis, cardiogenic shock, or a life-expectancy <1 year. Patients were randomized in a 1:1 ratio to receive ranolazine or placebo. The protocol (including the biomarker substudy) was approved by institutional review boards, and written consent was obtained from all patients, including for the biomarker substudy.

Biomarker Testing

Blood samples were collected at enrollment, followed by isolation of serum within 60 minutes of acquisition. Samples were stored in plastic cryovials at −20°C or colder at the enrolling site until shipped to the TIMI Clinical Trials Laboratory (Boston, MA) where they were maintained at −80°C or colder. All biomarker testing was performed in the TIMI Clinical Trials Laboratory by personnel masked to clinical outcomes and treatment allocation.

Beta-CTx was measured using the Roche Cobas 6000 e601 analyzer and the β-CrossLaps assay in serum samples with a functional sensitivity (ie, lowest concentration measured with a coefficient of variation <20%) of 0.070 ng/mL and a measuring range from 0.01 to 6.00 ng/mL.[14] Observed run-to-run coefficients of variation were 2.5% at 0.326 ng/mL and 1.7% at 0.830 ng/mL.

End Points

The primary end point for the present biomarker analysis was time to the composite of cardiovascular death or new or worsening HF. Worsening of HF was defined as re-hospitalization or prolongation of the index hospitalization (>24 hours) in an acute care facility primarily for the treatment of HF along with an objective sign of HF. Additional end points were the individual components of the composite end point, sudden cardiac death, and all-cause mortality. All end points were adjudicated by a masked clinical events committee.

Statistical Analysis

The baseline characteristics of this patient cohort stratified by beta-CTx quartiles were compared using the Kruskal–Wallis tests for continuous variables and the Chi-square test for categorical variables. To assess the trend across the quartiles, the Jonckheere-Terpstra trend test was used for continuous and the Cochran-Armitage Trend test for categorical variables. Correlations of the biomarkers (modeled as continuous variables) were examined using Spearman correlation coefficients. Plasma concentrations of C-terminal telopeptides as well as levels of the established biomarkers (hsTnT, NT-proBNP, and hsCRP) were categorized using quartiles for survival analyses. Beta-CTx was also included as a continuous variable for sensitivity analyses. Kaplan–Meier event rates at 12 months are reported. The Logrank test was used to compare the survival distribution. Adjusted hazard ratios (adj-HR) were determined using a Cox-proportional-hazards

regression model that included the following variables age, sex, history of diabetes mellitus, prior myocardial infarction, prior HF, revascularization during index hospitalization, TIMI risk score, and baseline estimated glomerular filtration rate (modeled as a categorical variable <45, 45 to <60, 60 to <90, and ≥90 mL/min per 1.73 m^2). We used a comprehensive model inclusive of variables considered a priori to be of clinical relevance to outcomes in ACS. As ≈33% of all subjects did not have information available for left ventricular ejection fraction (LVEF) at baseline, models adjusting also for LVEF were only performed as sensitivity analyses. The proportional hazards assumption was confirmed using statistical tests and visual inspection based on the scaled Schoenfeld residuals.[15] The discriminatory performance of each biomarker employed as continuous variables was assessed using C-statistics based on the Harrell method. In addition, reclassification analyses were performed using integrated discrimination improvement, and the net re-classification improvement at the event rate for each biomarker (results shown in Data S1).[16] The confidence intervals for integrated discrimination improvement were based on standard errors estimated from 300 bootstrap samples. All analyses were performed using SAS (Version 9.4; SAS Institute, USA). *P* values (2-tailed) <0.05 were considered to indicate statistical significance. Because of the exploratory nature of this analysis no adjustments for multiple testing were performed.

Results

Study Population

Baseline beta-CTx levels were measured in 4094 patients. Of these patients, 2637 (64.4%) were men and the median age was 64 (interquartile range [IQR]: 56–72) years (Table). Over a median follow-up of 345 days (IQR: 235–458), 167 patients were hospitalized for HF and cardiovascular death occurred in 187 patients, 78 of whom died of sudden cardiac death.

Biomarker Levels at Baseline

The median of beta-CTx measured at baseline was 0.259 ng/mL (IQR: 0.174–0.389 ng/mL). Patients with higher beta-CTx serum levels were more likely to be women, have a history of HF, lower estimated glomerular filtration rate levels, and a

Table. Baseline Characteristics for the Total Study Population and Stratified by Quartiles of C-Terminal Telopeptidase

	Overall (n=4094)	Quartile 1 (n=1033)	Quartile 2 (n=1021)	Quartile 3 (n=1017)	Quartile 4 (n=1023)	*P* Trend
Age (y), median (IQR)	64 (56–72)	62 (55–70)	63 (55–70)	64 (55–71)	67 (58–74)	<0.001
Male sex, n (%)	2637 (64)	745 (72.1)	715 (70.0)	648 (63.7)	529 (51.7)	<0.001
Creatinine clearance (mL/min), median (IQR)	83 (64–106)	88.2 (68–112)	85.7 (67–108)	84.1 (65–105)	73.0 (54–96)	<0.001
Coronary revascularization, n (%)	1084 (26.5)	285 (27.6)	272 (26.7)	283 (27.9)	244 (23.9)	0.31
Prior myocardial infarction, n (%)	1439 (35.5)	356 (34.8)	371 (36.6)	345 (34.3)	367 (36.5)	0.67
History of heart failure, n (%)	869 (21.2)	172 (16.7)	214 (21.0)	201 (19.8)	282 (27.6)	<0.001
eGFR, median (IQR)	74.3 (60.6–88.9)	76.0 (64.6–90.7)	74.9 (61.9–89.3)	75.2 (60.7–89.1)	69.4 (54.3–85.1)	<0.001
LVEF, median (IQR)	55 (47–60)	56 (49–60)	55 (46–60)	55 (47–60)	55 (45–60)	0.009
LVEF <40%, n (%)	236 (8.6)	45 (7.2)	64 (9.5)	50 (7.0)	77 (10.5)	0.13
Diabetes mellitus, n (%)	1327 (32.4)	422 (40.9)	321 (31.4)	303 (29.8)	281 (27.5)	<0.001
TIMI risk score, median (IQR)	3 (3–4)	3 (2–4)	3 (3–4)	3 (3–4)	4 (3–5)	<0.001
≤2, n (%)	998 (24.4)	289 (28.0)	247 (24.2)	254 (25.0)	208 (20.3)	
3 to 4, n (%)	2201 (53.8)	518 (50.1)	574 (56.2)	554 (54.5)	555 (54.3)	
≥5, n (%)	895 (21.9)	226 (21.9)	200 (19.6)	209 (20.6)	260 (25.4)	
ACE inhibitors, n (%)	2969 (72.5)	729 (70.6)	729 (71.4)	761 (74.8)	750 (73.3)	0.062
Beta-blockers, n (%)	3969 (90.3)	944 (91.4)	912 (89.3)	928 (91.2)	912 (89.1)	0.25
Statin, n (%)	3221 (78.7)	870 (84.2)	833 (81.6)	796 (78.3)	722 (70.6)	<0.001
hsCRP (mg/L), median (IQR)	5.5 (2.5–12.6)	5.5 (2.4–14.4)	5.6 (2.6–12.2)	5.7 (2.7–12.6)	5.2 (2.1–11.2)	0.089
hsTnT (pg/mL), median (IQR)	60 (10–305)	53 (9–299)	58 (10–279)	68 (9–342)	59 (10–322)	0.22
NT-proBNP (pg/mL), median (IQR)	354 (125–966)	262 (98–676)	306 (115–815)	369 (136–954)	561 (165–1522)	<0.001

ACE indicates angiotensin-converting enzyme; eGFR, estimated glomerular filtration rate; IQR, interquartile range; LVEF, left ventricular ejection fraction; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

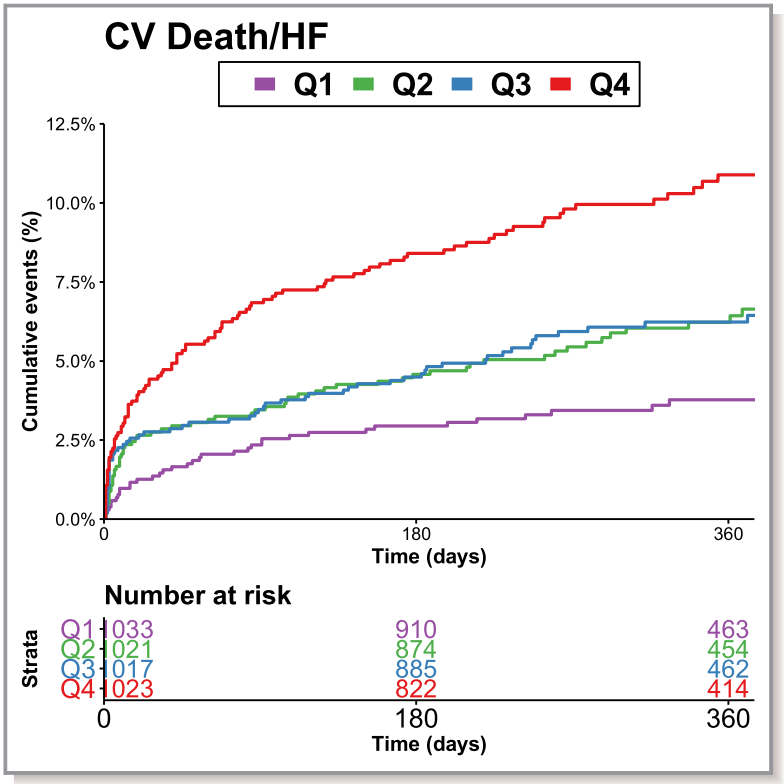


Figure 1. Kaplan–Meier curves for the composite of cardiovascular death and new or worsening heart failure stratified by quartiles of C-terminal telopeptidase. Logrank $P<0.001$. CV indicates cardiovascular; HF, heart failure.

higher TIMI risk score but less likely to suffer from diabetes mellitus (Table). Beta-CTx correlated weakly with NT-proBNP ($r=0.17$, $P<0.001$), while conversely a weak negative correlation with creatinine clearance ($r=-0.19$, $P<0.001$) and a weak negative correlation with LVEF ($r=-0.05$, $P=0.008$) was observed (Table S1). No correlation was found between beta-CTx and hsTnT ($r=0.02$, $P=0.20$), or hsCRP ($r=-0.03$, $P=0.09$).

Biomarker Levels and Cardiovascular Outcomes

Higher beta-CTx serum levels were associated with increasing Kaplan-Meier event rates of cardiovascular death/HF at 12 months (quartile (Q)1: 3.8%, Q2: 6.2%, Q3: 6.2%, Q4 10.9%; Logrank $P<0.0001$, Figure 1). This graded relationship was also observed for the individual components and for sudden cardiac death (Q4 versus Q1: cardiovascular death 7.29% versus 1.81%; HF 6.67% versus 2.71%; sudden cardiac death 2.90% versus 1.18%, all Logrank $P<0.0001$; Figure 2). The event rates stratified by both beta-CTx and left ventricular function, NT-proBNP or hsTnT, respectively, indicated beta-CTx to be of complementary nature with both markers (Figure 3; Figures S1 through S3).

The unadjusted HR (Q4 versus Q1) for cardiovascular death/HF were 3.02 (95% CI: 2.10–4.35), for cardiovascular death 4.01 (95% CI: 2.45–6.56), and for HF 2.61 (95% CI: 1.67–4.09). After multivariable adjustment for clinical variables, patients with beta-CTx serum concentrations in Q4 were associated with a significantly higher risk of cardiovascular death/HF (adj. HR 2.22 [95% CI: 1.50–3.27]; Figure 4). The association between beta-CTx concentrations and cardiovascular outcomes tended to increase with higher quartiles (all P-trend <0.05; Figure S4). Further adjustment for LVEF was performed in a sensitivity analysis showing similar results (adj. HR 2.21 [95% CI: 1.32–3.69]; Figure S5). This relationship between beta-CTx and cardiovascular outcomes remained significant when including beta-CTx as a continuous variable (Figure S6). In terms of discrimination, beta-CTx performed moderately well as a sole risk marker (area under the curve for cardiovascular death/HF: 0.61 [95% CI: 0.58–0.65]; Data S1, Table S2).

In a multimarker approach adjusted for clinical variables and the 3 biomarkers NT-proBNP, hsTnT, and hsCRP, beta-CTx in the top quartile remained independently associated with cardiovascular death/HF (adj. HR 1.98 [95% CI: 1.34–2.93]) and the individual components (cardiovascular death: adj. HR 2.29 [95% CI: 1.35–3.89], HF: adj. HR 1.73 [95% CI: 1.07–2.81]; Figure 5). The variance inflation factors for each biomarker were low (<2.2) suggesting that multicollinearity

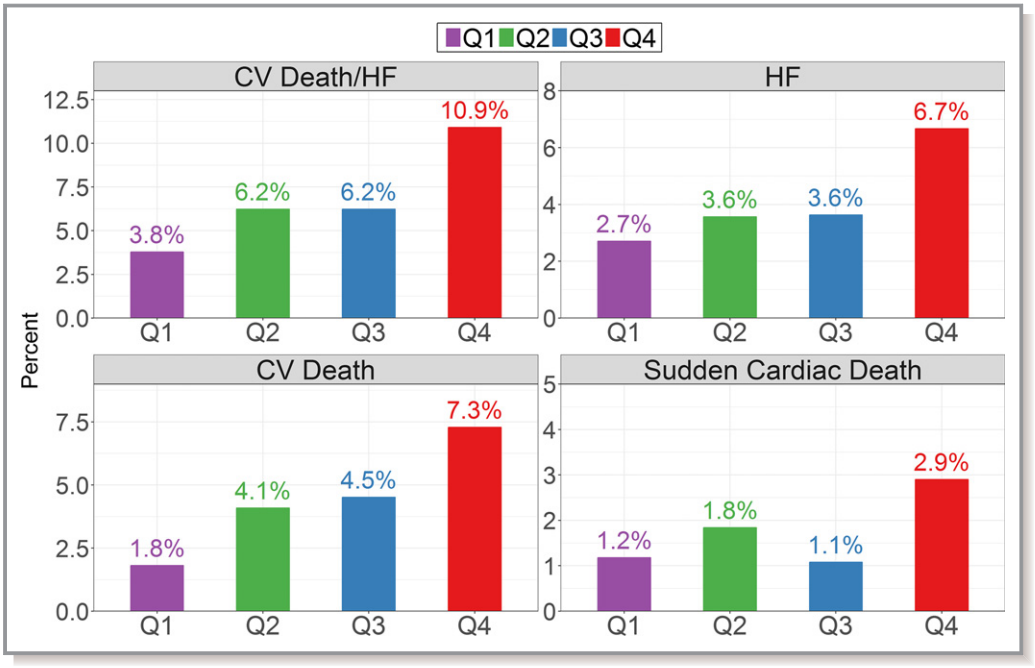


Figure 2. Kaplan–Meier event rates at 12 months by quartiles of C-terminal telopeptidase for the composite of cardiovascular death and new or worsening heart failure, its individual components, and sudden cardiac death. Logrank $P<0.001$ for all tested outcomes. CV indicates cardiovascular; HF, heart failure.

was low. These results remained qualitatively similar when including the biomarkers separately.

Discussion

This study examined the role of a marker of collagen turnover (beta-CTx) in patients with non–ST-elevation acute coronary syndrome. Higher concentrations of beta-CTx were significantly associated with cardiovascular death and HF even after multivariable adjustment for clinical variables and established biomarkers, including hsTnT and NT-proBNP.

Although mortality after acute MI has declined substantially over the past few decades, patients who have had an MI are at increased risk for HF and sudden cardiac death.[17] A number of clinical features have been identified as possible risk factors for the development of fatal arrhythmia following an acute MI.[18] In this regard, a reduced left ventricular ejection fraction and HF are among the most powerful predictors.[19,20] However, early identification of patients at the highest risk of sudden cardiac

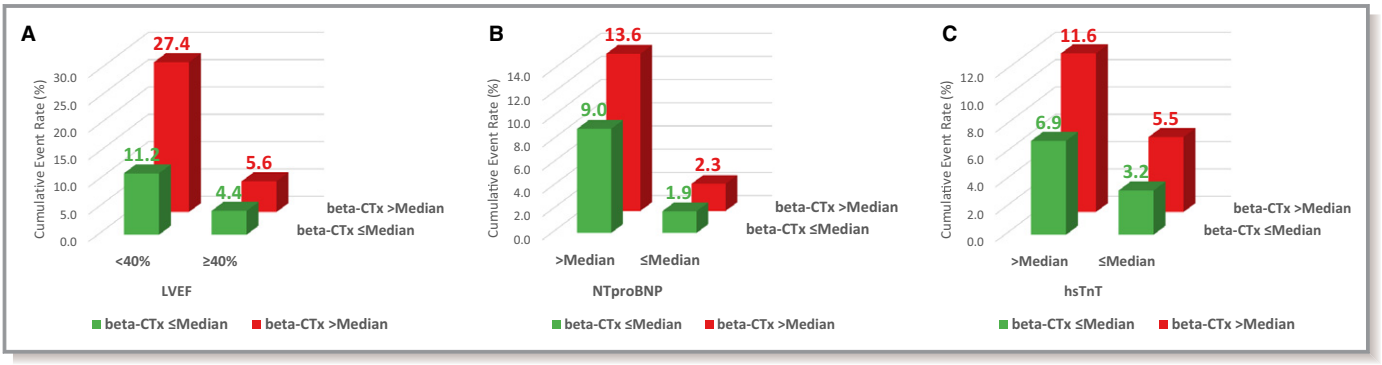


Figure 3. Kaplan–Meier event rates at 12 months stratified by the median of C-terminal telopeptidase (beta-CTx) and (**A**) NT-proBNP or (**B**) hsTnT, respectively, for the composite of cardiovascular death and new or worsening heart failure. **A**, LVEF <40%: beta-CTx ≤ median vs > median; Logrank $P=0.005$, LVEF ≥40%: beta-CTx ≤ median vs > median; Logrank $P=0.095$. **B**, NT-proBNP > median: beta-CTx ≤ median vs > median; Logrank $P=0.002$, NT-proBNP ≤ median: beta-CTx ≤ median vs > median; Logrank $P=0.54$, **C**, hsTnT > median: beta-CTx ≤ median vs > median; Logrank $P<0.001$. hsTnT ≤ median: beta-CTx ≤ median vs > median; Logrank $P=0.014$. beta-CTx indicates C-terminal telopeptidase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hsTnT, high-sensitivity cardiac troponin T.

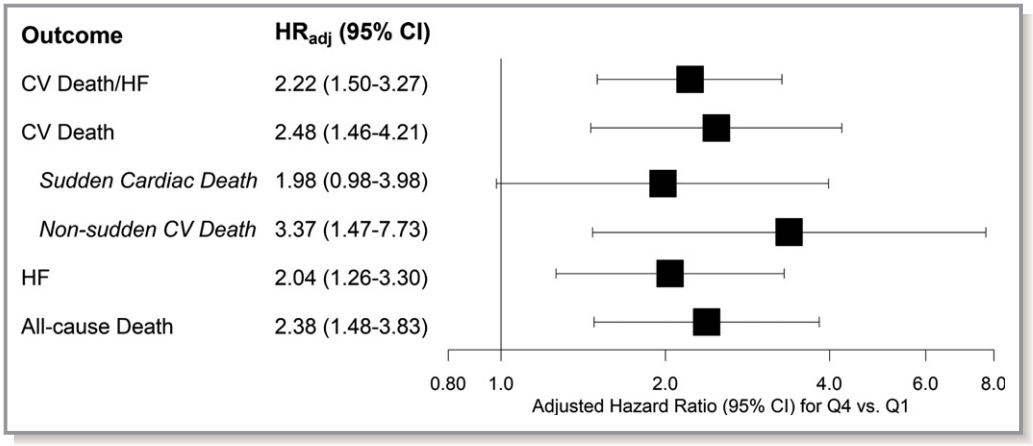


Figure 4. Adjusted hazard for beta-CTx serum concentrations in the top vs lowest quartile for the composite of new or worsening heart failure (HF) and cardiovascular (CV) death, its individual components and sudden cardiac death. HR_{adj} indicates adjusted hazard ratio.

death can be challenging[17] and non-invasive identification might facilitate selection of patients who are early candidates for device therapies or other approaches to mitigating the risk of sudden cardiac death.[20]

In addition, despite the substantial progress made in the management of HF,[21] there is an unmet need for improved treatment strategies. Therapeutics that target cardiac remodeling by influencing the expression of collagen have been

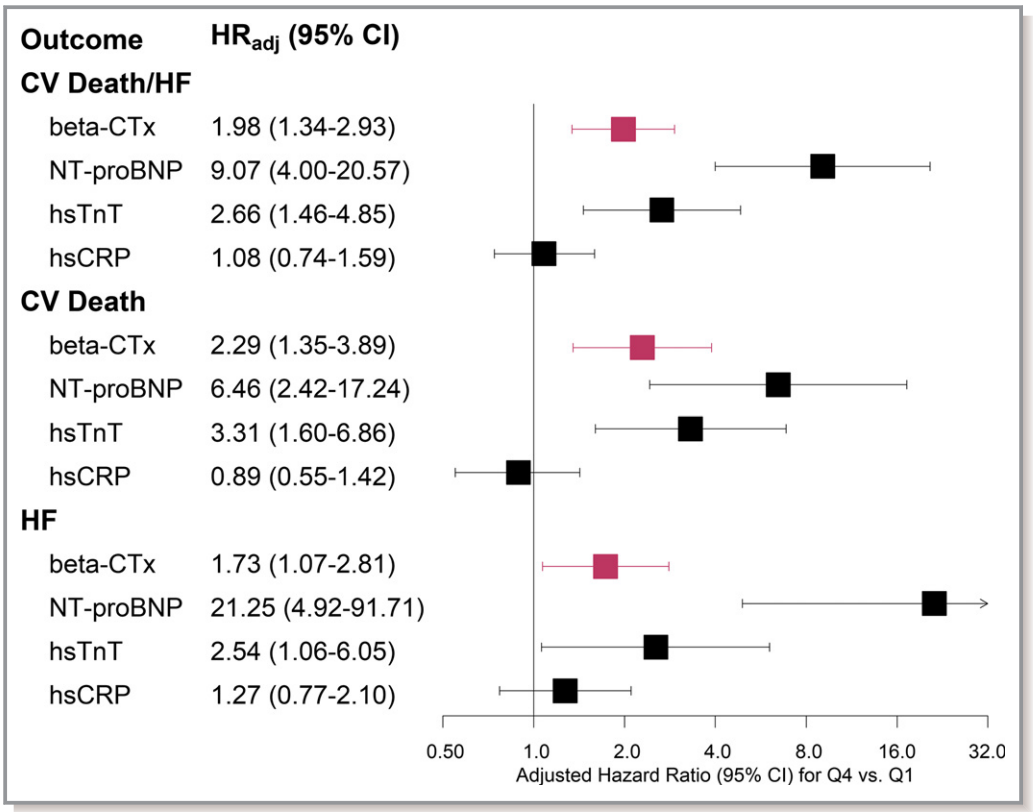


Figure 5. Adjusted multimarker model for the composite of cardiovascular death and new or worsening heart failure, and its individual components. Models were adjusted for age, prior myocardial infarction, prior heart failure, revascularization during index hospitalization, TIMI risk score, estimated glomerular filtration rate at baseline, NT-proBNP, hsTnT, and hsCRP. beta-CTx indicates C-terminal telopeptidase; CV, cardiovascular; HF, heart failure; HR_{adj}, adjusted hazard ratio; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

identified as potential novel treatment strategies.[22] The molecular mechanisms of fibrosis are complex and not completely understood, but the equilibrium of synthesis and degradation of collagen appears to be paramount. The predominance of accumulation over degradation of collagen in the myocardium results in cardiac fibrosis leading to impaired diastolic function and potentially contributing to the genesis of life-threatening arrhythmias. Contrary, increased degradation over synthesis may result in the disruption of the matrix and be responsible for ventricular dilation and reduced systolic function. Patients with MI may develop both phenotypes with focal or diffuse patterns.

The gold standard for the assessment of presence of fibrosis with cardiac biopsies is an invasive method and object to sampling error. Routine non-invasive measurement using cardiac magnetic resonance imaging is constrained by cost and availability. Therefore, biomarkers of collagen turnover offer an attractive approach as potential indicators of myocardial fibrosis. Markers of increased synthesis such as C-terminal propeptide of procollagen type I[2,23,24] and N-terminal propeptide of procollagen type III[25,26] have been associated with adverse outcomes of HF in multiple studies. However, conflicting data exist on markers of degradation.[26] Beta-CTx serves as a specific marker for the degradation of mature type I collagen and smaller studies suggested that beta-CTx may be associated with unfavorable cardiovascular outcomes such as atrial fibrillation, HF, or cardiovascular death.[27,28] Recently, a study that followed 3187 apparently healthy individuals over a median time of 13 years found that high levels of beta-CTx were associated with the incidence of HF with preserved ejection fraction but not with HF with reduced ejection fraction.[29] The present analysis in a population with acute ischemic heart disease and robust adjustment for potential confounding variables adds evidence to the current understanding. Because of its moderate discriminatory performance, beta-CTx may be limited as a new biomarker for use alone in risk stratification but it may be used in conjunction with other biomarkers or clinical risk indicators. In addition, our findings reinforce the potential pathobiological importance of collagen in the development of HF. The correlation coefficients between beta-CTx and cardiac, or inflammatory biomarkers are low suggesting that targeting collagen degradation represents an important, prognostic indicator that is complementary to hemodynamic stress, myocardial stretch, myocardial injury, or inflammation.

Limitations

The application of biomarkers of collagen turnover are limited by the abundance of collagen and the lack of cardiac specificity. Despite multivariable adjustment, our study may be subject to residual confounding. As approximately one third of the patient cohort did not have LVEF information available, models adjusting LVEF were only performed as sensitivity analyses in a subset of patients but provided similar results. In addition, markers of collagen synthesis were not available, which in combination with beta-CTx, a marker of degradation, may add further prognostic value or pathobiological insight. Furthermore, the present study does not provide the opportunity to correlate the accumulation of fibrous tissue with objective results from tissue biopsies or cardiac magnetic resonance imaging. We do not have serial measurements of beta-CTx to examine quantification of the extent of fibrosis over time. Because of the exploratory design, no corrections for multiple testing were performed.

Conclusion

Beta-CTx, a marker of collagen turnover, was associated with cardiovascular death/HF and its individual components in patients with non–ST-elevation acute coronary syndrome and, in addition to providing pathobiological insight, may prove to be useful when added to other approaches for estimation of risk in these patients.

Sources of Funding

Research reported in this manuscript was supported by National Institutes of Health National Center for Advancing Translational Sciences of the National Institutes of Health under award number: UH3TR000901. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Zelniker is supported by the Deutsche Forschungsgemeinschaft (DFG, ZE 1109/1-1). The MERLIN-TIMI 36 trial was funded by CV Therapeutics. Reagents for NTproBNP and hsTnT were supported by Roche Diagnostics.

Disclosures

Dr Zelniker was supported by a research grant of the Deutsche Forschungsgemeinschaft (DFG, ZE 1109/1-1). Dr Jarolim reports research support through his institution from Abbott Laboratories, AstraZeneca, LP, Daiichi-Sankyo, Inc., GlaxoSmithKline, Merck & Co., Inc., Roche Diagnostics Corporation, Takeda Global Research and Development Center, and Waters Technologies Corporation; and consulting fees from Roche Diagnostics Corporation. Dr Scirica reports research grants via Brigham and Women's Hospital from AstraZeneca, Eisai, Novartis, and Merck. Consulting fees from Allergan, AstraZeneca, Boehringer Ingelheim, Covance, Eisai, Elsevier Practice Update Cardiology, GlaxoSmithKline, Lexicon, Merck, NovoNordisk, Sanofi, and equity in Health [at] Scale. For the

subject matter of this manuscript, Dr Braunwald reports grant support to his institution from CV Therapeutics (now Gilead Sciences) for his role as Chairman of the MERLIN-TIMI 36 trial. Dr Das reports the following disclosures: (1) Founding member of LQT Therapeutics and Dyrnamix, neither of which played any role in this study, (2) Novo Nordisk (travel) as visiting scientist. Dr Sabatine reports research grant support through Brigham and Women's Hospital from Abbott Laboratories; Amgen; AstraZeneca; Bayer; Daiichi-Sankyo; Eisai; Gilead; GlaxoSmithKline; Intarcia; Janssen Research and Development; Medicines Company; MedImmune; Merck; Novartis; Poxel; Pfizer; Quark Pharmaceuticals; Roche Diagnostics; Takeda. (All >$10 000 per year) and consulting for Alnylam; Amgen; AstraZeneca; Bristol-Myers Squibb; CVS Caremark; DalCor; Dyrnamix; Esperion; IFM Therapeutics; Intarcia; Ionis; Janssen Research and Development; Medicines Company; MedImmune; Merck; MyoKardia; Novartis (all ≤$10 000 per year except Amgen, Esperion & Ionis). Dr Morrow reports grants to the TIMI Study Group from Abbott Laboratories, Amgen, Astra Zeneca, BRAHMS, Eisai, Glaxosmithkline, Medicines Co, Merck, Novartis, Pfizer, Roche Diagnostics, Quark, Takeda and consultant fees from Abbott Laboratories, Aralez, Astra Zeneca, Bayer Pharma, InCardia, Pfizer, and Roche Diagnostics. Dr Park has no disclosures to report.

References

1. Vazir A, Fox K, Westaby J, Evans MJ, Westaby S. Can we remove scar and fibrosis from adult human myocardium? *Eur Heart J*. 2018;40:960–966.
2. Querejeta R, Lopez B, Gonzalez A, Sanchez E, Larman M, Martinez Ubago JL, Diez J. Increased collagen type I synthesis in patients with heart failure of hypertensive origin: relation to myocardial fibrosis. *Circulation*. 2004;110:1263–1268.
3. Lopez B, Gonzalez A, Diez J. Circulating biomarkers of collagen metabolism in cardiac diseases. *Circulation*. 2010;121:1645–1654.
4. Lopez B, Ravassa S, Gonzalez A, Zubillaga E, Bonavila C, Berges M, Echegaray K, Beaumont J, Moreno MU, San Jose G, Larman M, Querejeta R, Diez J. Myocardial collagen cross-linking is associated with heart failure hospitalization in patients with hypertensive heart failure. *J Am Coll Cardiol*. 2016;67:251–260.
5. Di Marco A, Anguera I, Schmitt M, Klem I, Neilan TG, White JA, Sramko M, Masci PG, Barison A, McKenna P, Mordi I, Haugaa KH, Leyva F, Rodriguez Capitan J, Satoh H, Nabeta T, Dallaglio PD, Campbell NG, Sabate X, Cequier A. Late gadolinium enhancement and the risk for ventricular arrhythmias or sudden death in dilated cardiomyopathy: systematic review and meta-analysis. *JACC Heart Fail*. 2017;5:28–38.
6. Brower GL, Gardner JD, Forman MF, Murray DB, Voloshenyuk T, Levick SP, Janicki JS. The relationship between myocardial extracellular matrix remodeling and ventricular function. *Eur J Cardiothorac Surg*. 2006;30:604–610.
7. Collier P, Watson CJ, van Es MH, Phelan D, McGorrian C, Tolan M, Ledwidge MT, McDonald KM, Baugh JA. Getting to the heart of cardiac remodeling; how collagen subtypes may contribute to phenotype. *J Mol Cell Cardiol*. 2012;52:148–153.
8. Norton GR, Tsotetsi J, Trifunovic B, Hartford C, Candy GP, Woodiwiss AJ. Myocardial stiffness is attributed to alterations in cross-linked collagen rather than total collagen or phenotypes in spontaneously hypertensive rats. *Circulation*. 1997;96:1991–1998.
9. Koshy SK, Reddy HK, Shukla HH. Collagen cross-linking: new dimension to cardiac remodeling. *Cardiovasc Res*. 2003;57:594–598.
10. Badenhorst D, Maseko M, Tsotetsi OJ, Naidoo A, Brooksbank R, Norton GR, Woodiwiss AJ. Cross-linking influences the impact of quantitative changes in myocardial collagen on cardiac stiffness and remodelling in hypertension in rats. *Cardiovasc Res*. 2003;57:632–641.
11. Lopez B, Gonzalez A, Ravassa S, Beaumont J, Moreno MU, San Jose G, Querejeta R, Diez J. Circulating biomarkers of myocardial fibrosis: the need for a reappraisal. *J Am Coll Cardiol*. 2015;65:2449–2456.
12. Morrow DA, Scirica BM, Karwatowska-Prokopczuk E, Skene A, McCabe CH, Braunwald E; MERLIN-TIMI 36 Investigators. Evaluation of a novel anti-ischemic agent in acute coronary syndromes: design and rationale for the Metabolic Efficiency with Ranolazine for Less Ischemia in non-ST-elevation acute coronary syndromes (MERLIN)-TIMI 36 trial. *Am Heart J*. 2006;151:1186.e1–9.
13. Morrow DA, Scirica BM, Karwatowska-Prokopczuk E, Murphy SA, Budaj A, Varshavsky S, Wolff AA, Skene A, McCabe CH, Braunwald E; MERLIN-TIMI 36 Trial Investigators. Effects of ranolazine on recurrent cardiovascular events in patients with non-ST-elevation acute coronary syndromes: the MERLIN-TIMI 36 randomized trial. *JAMA*. 2007;297:1775–1783.
14. Garnero P, Borel O, Delmas PD. Evaluation of a fully automated serum assay for C-terminal cross-linking telopeptide of type I collagen in osteoporosis. *Clin Chem*. 2001;47:694–702.
15. Schoenfeld D. Partial residuals for the proportional hazards regression model. *Biometrika*. 1982;69:239–241.
16. Pencina MJ, Steyerberg EW, D'Agostino RB Sr. Net reclassification index at event rate: properties and relationships. *Stat Med*. 2017;36:4455–4467.
17. Solomon SD, Zelenkofske S, McMurray JJ, Finn PV, Velazquez E, Ertl G, Harsanyi A, Rouleau JL, Maggioni A, Kober L, White H, Van de Werf F, Pieper K, Califf RM, Pfeffer MA; Valsartan in Acute Myocardial Infarction Trial (VALIANT) Investigators. Sudden death in patients with myocardial infarction and left ventricular dysfunction, heart failure, or both. *N Engl J Med*. 2005;352:2581–2588.
18. Multicenter Postinfarction Research G. Risk stratification and survival after myocardial infarction. *N Engl J Med*. 1983;309:331–336.
19. Adabag AS, Therneau TM, Gersh BJ, Weston SA, Roger VL. Sudden death after myocardial infarction. *JAMA*. 2008;300:2022–2029.
20. Al-Khatib SM, Stevenson WG, Ackerman MJ, Bryant WJ, Callans DJ, Curtis AB, Deal BJ, Dickfeld T, Field ME, Fonarow GC, Gillis AM, Granger CB, Hammill SC, Hlatky MA, Joglar JA, Kay GN, Matlock DD, Myerburg RJ, Page RL. 2017 AHA/ACC/HRS guideline for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Circulation*. 2018;138:e272–e391.
21. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR, PARADIGM-HF Investigators and Committees. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med*. 2014;371:993–1004.
22. Yamagami K, Oka T, Wang Q, Ishizu T, Lee JK, Miwa K, Akazawa H, Naito AT, Sakata Y, Komuro I. Pirfenidone exhibits cardioprotective effects by regulating myocardial fibrosis and vascular permeability in pressure-overloaded hearts. *Am J Physiol Heart Circ Physiol*. 2015;309:H512–H522.
23. Querejeta R, Varo N, Lopez B, Larman M, Artinano E, Etayo JC, Martinez Ubago JL, Gutierrez-Stampa M, Emparanza JI, Gil MJ, Monreal I, Mindan JP, Diez J. Serum carboxy-terminal propeptide of procollagen type I is a marker of myocardial fibrosis in hypertensive heart disease. *Circulation*. 2000;101:1729–1735.
24. Lopez B, Querejeta R, Gonzalez A, Sanchez E, Larman M, Diez J. Effects of loop diuretics on myocardial fibrosis and collagen type I turnover in chronic heart failure. *J Am Coll Cardiol*. 2004;43:2028–2035.
25. Klappacher G, Franzen P, Haab D, Mehrabi M, Binder M, Plesch K, Pacher R, Grimm M, Pribill I, Eichler HG, Glogar D. Measuring extracellular matrix turnover in the serum of patients with idiopathic or ischemic dilated cardiomyopathy and impact on diagnosis and prognosis. *Am J Cardiol*. 1995;75:913–918.
26. Izawa H, Murohara T, Nagata K, Isobe S, Asano H, Amano T, Ichihara S, Kato T, Ohshima S, Murase Y, Iino S, Obata K, Noda A, Okumura K, Yokota M. Mineralocorticoid receptor antagonism ameliorates left ventricular diastolic dysfunction and myocardial fibrosis in mildly symptomatic patients with idiopathic dilated cardiomyopathy: a pilot study. *Circulation*. 2005;112:2940–2945.
27. Ravassa S, Lopez B, Querejeta R, Echegaray K, San Jose G, Moreno MU, Beaumont FJ, Gonzalez A, Diez J. Phenotyping of myocardial fibrosis in hypertensive patients with heart failure. Influence on clinical outcome. *J Hypertens*. 2017;35:853–861.
28. Duprez DA, Gross MD, Sanchez OA, Kizer JR, Ix JH, Lima J, Tracy RP, Jacobs DR Jr. Collagen turnover markers in relation to future cardiovascular and noncardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. *Clin Chem*. 2017;63:1237–1247.
29. Duprez DA, Gross MD, Kizer JR, Ix JH, Hundley WG, Jacobs DR Jr. Predictive value of collagen biomarkers for heart failure with and without preserved ejection fraction: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Heart Assoc*. 2018;7:e007885. DOI: 10.1161/JAHA.117.007885.

SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Discriminatory performance and reclassification analyses

Beta-CTx performed moderately well in discrimination of all tested outcomes (CV death/HF: AUC 0.61 [95% CI 0.58-0.65]; CV death: AUC 0.64 [95% CI 0.60-0.68]; HF: AUC 0.61 [95% CI 0.56-0.66]; sudden cardiac death: AUC 0.61 [95% CI 0.54-0.68]) with qualitatively similar performance compared with hsTnT (CV death/HF: AUC 0.66 [95% CI 0.63-0.69]; CV death: AUC 0.66 [95% CI 0.62-0.70]; HF: AUC 0.67 [95% CI 0.63-0.71]; sudden cardiac death: AUC 0.64 [95% CI 0.0.58-0.70]) and hsCRP (CV death/HF: AUC 0.61 [95% CI 0.57-0.64]; CV death: AUC 0.59 [95% CI 0.55-0.64]; HF: AUC 0.62 [95% CI 0.58-0.66]; sudden cardiac death: AUC 0.62 [95% CI 0.56-0.68]). Only NT-proBNP clearly outperformed all tested biomarkers exhibiting excellent accuracy (CV death/HF: AUC 0.78 [95% CI 0.75-0.80]; CV death: AUC 0.78 [95% CI 0.75-0.82], HF: AUC 0.80 [95% CI 0.77-0.84]; sudden cardiac death: AUC 0.77 [95% CI 0.72-0.82]).

Reclassification analyses indicated an improvement in risk discrimination for the CV death/HF when including beta-CTx to a model including clinical variables and biomarkers (NRI at the event rate: 0.0207 (0.0002, 0.0412), continuous NRI: 0.1435 (0.0393, 0.2476), IDI 0.0045 (0.0006, 0.0085); Table S2). Similarly, the NR at the event rate indicated a significant improvement in reclassification for CV death (NRI at the event rate: 0.0214 (0.0065, 0.0363)) and all-cause mortality (NRI at the event rate: 0.1477 (0.1111, 0.1842); Table S2).

Table S1. Correlation matrix of all tested biomarkers displaying the Spearman correlation coefficients and respective p-values for C-terminal telopeptide, NT-proBNP, high-sensitivity troponin T, creatinine clearance, and the left ventricular ejection fraction.

	NT-proBNP	hsTnT	hsCRP	CrCl	LVEF
Beta-CTX	0.17	0.02	-0.03	-0.19	-0.05
	<0.001	0.20	0.09	<0.001	0.008
NT-proBNP		0.58	0.30	-0.37	-0.31
		<0.001	<0.001	<0.001	<0.001
hsTnT			0.32	-0.07	-0.16
			<0.001	<0.001	<0.001
hsCRP				-0.01	-0.11
				0.41	<0.001
CrCl					0.04
					0.026

beta-CTX = C-terminal telopeptide, CrCl = creatinine clearance, LVEF = left ventricular ejection fraction, NT-proBNP = N-terminal pro B-type natriuretic peptide, hsCRP = high sensitivity C-reactive protein, hsTnT = high-sensitivity troponin T

Table S2. Reclassification Analyses.

	NRI at the Event Rate	Continuous NRI	IDI
CV Death/HF			
Model 1 ± beta-CTx	**0.0207 (0.0002, 0.0412)**	**0.1435 (0.0393, 0.2476)**	**0.0045 (0.0006, 0.0085)**
Model 2 ± beta-CTx	-0.0079 (-0.0231, 0.0073)	-0.0288 (-0.1333, 0.0757)	-0.0002 (-0.0025, 0.0021)
CV Death			
Model 1 ± beta-CTx	**0.0214 (0.0065, 0.0363)**	**0.1617 (0.0481, 0.2754)**	0.0028 (-0.0013, 0.0069)
Model 2 ± beta-CTx	-0.0039 (-0.0173, 0.0095)	0.0402 (-0.0735, 0.1539)	-0.0003 (-0.0026, 0.0020)
Sudden Cardiac Death			
Model 1 ± beta-CTx	0.0244 (-0.0063, 0.0550)	**0.3155 (0.1661, 0.4649)**	**0.0040 (0.0002, 0.0077)**
Model 2 ± beta-CTx	-0.0026 (-0.0085, 0.0034)	**0.1911 (0.0402, 0.3420)**	0.0022 (-0.0015, 0.0059)
Heart Failure			
Model 1 ± beta-CTx	-0.0022 (-0.0320, 0.0275)	0.1102 (-0.0314, 0.2518)	**0.0049 (0.0008, 0.0090)**
Model 2 ± beta-CTx	-0.0065 (-0.0237, 0.0106)	-0.0611 (-0.2028, 0.0806)	0.0009 (-0.0010, 0.0028)
All-cause Mortality			
Model 1 ± beta-CTx	**0.1477 (0.1111, 0.1842)**	**0.1890 (0.0807, 0.2974)**	0.0034 (-0.0008, 0.0076)
Model 2 ± beta-CTx	-0.0068 (-0.0231, 0.0094)	0.0741 (-0.0343, 0.1826)	-0.0000 (-0.0027, 0.0027)

Model 1: age, sex, history of diabetes, prior MI, prior HF, revascularization during index hospitalization, TIMI risk score, and eGFR (modelled as a categorical variable <45, 45-<60, 60-<90, and ≥90 ml/min/1.73m^2)

Model 2: Model 1 + hsCRP, NT-proBNP, and hsTnT

Figure S1. Kaplan Meier event rates at 12 months stratified by quartiles of C-terminal telopeptidase (beta-CTx) and [A] NT-proBNP or [B] hsTnT, respectively, for the composite of cardiovascular (CV) death and hospitalization for heart failure (HF).

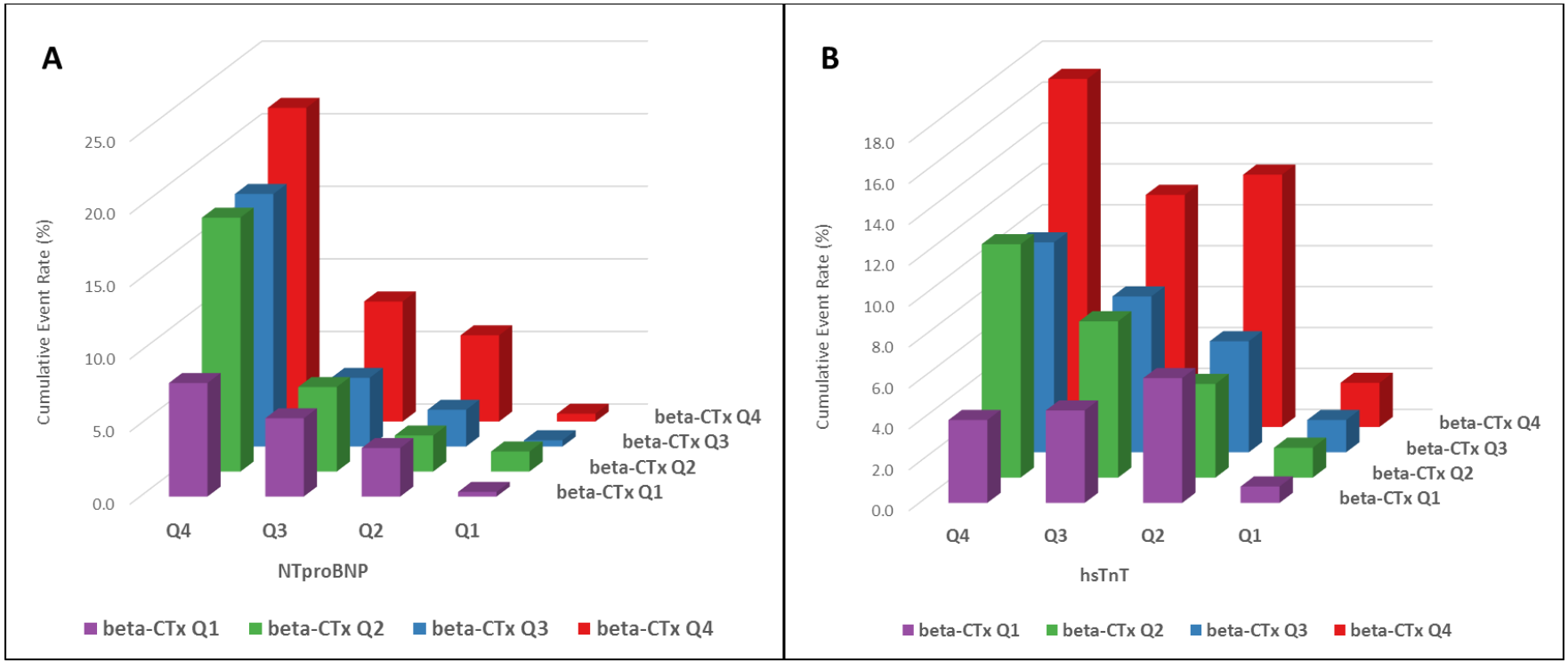


Figure S2. Kaplan Meier event rates at 12 months stratified by the median of C-terminal telopeptidase (beta-CTx) and [A] NT-proBNP or [B] hsTnT, respectively, for the composite of cardiovascular death and hospitalization for heart failure in patients with LVEF≥40%.

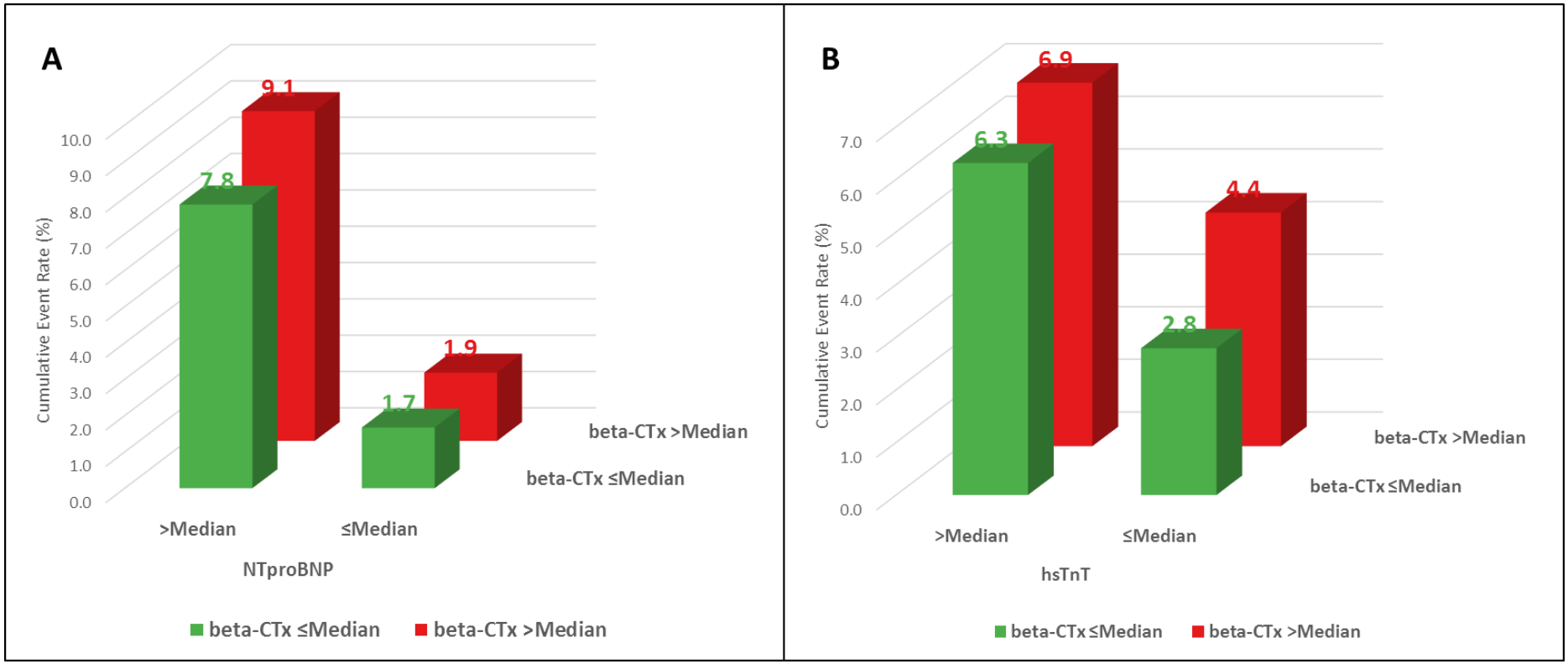


Figure S3. Kaplan Meier event rates at 12 months stratified by the median of C-terminal telopeptidase (beta-CTx) and [A] NT-proBNP or [B] hsTnT, respectively, for the composite of cardiovascular death and hospitalization for heart failure in patients with LVEF<40%.

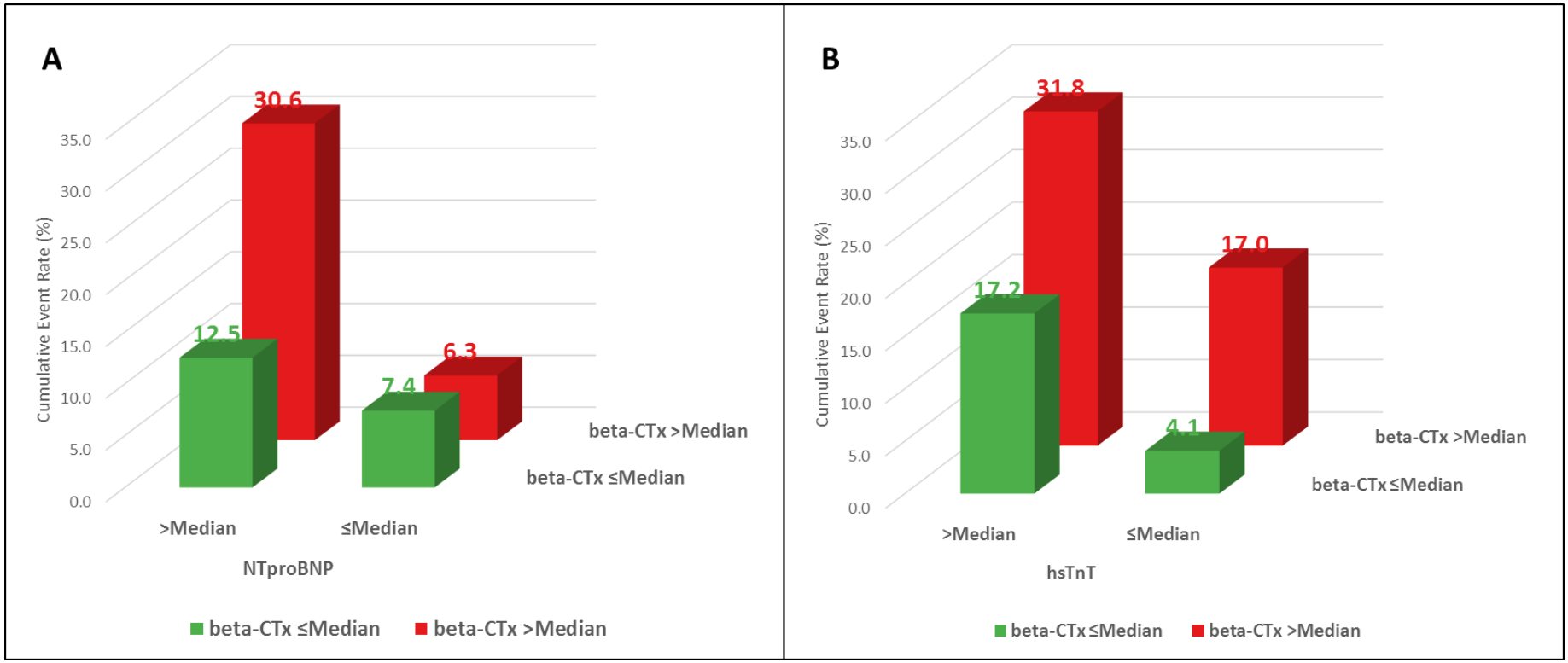


Figure S4. Adjusted hazard for quartiles of beta-CTx serum concentrations and the composite of hospitalization for heart failure (HF) and cardiovascular (CV) death, its individual components and all-cause death.

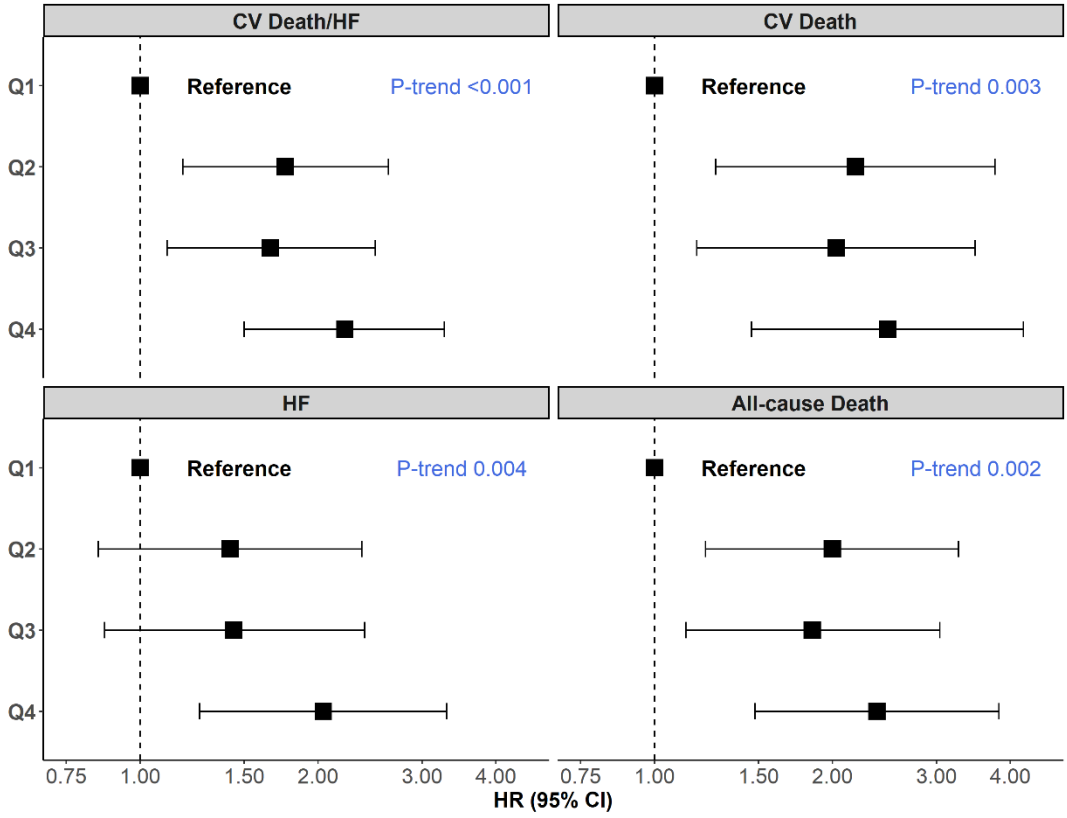
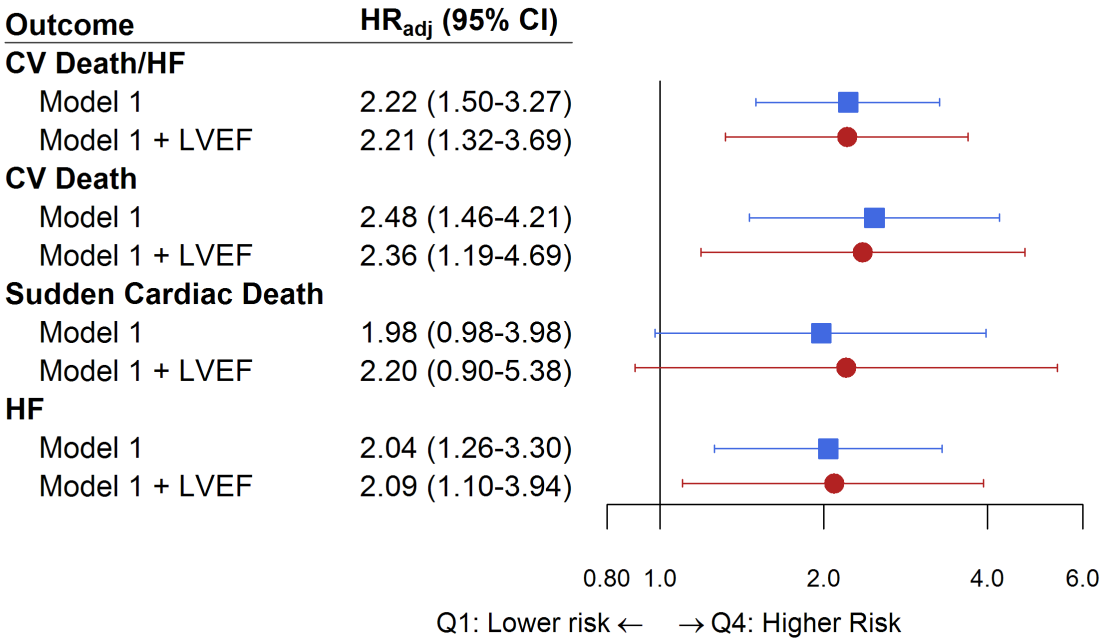


Figure S5. Sensitivity analyses examining adjusted hazard ratios for beta-CTX serum concentrations in the top versus lowest quartile for the composite of cardiovascular (CV) death or hospitalization for heart failure (HF), and its individual components in a subset with available left ventricular ejection fraction (LVEF).



Model 1: age, prior myocardial infarction, prior heart failure, revascularization during index hospitalization, TIMI risk score, and eGFR at baseline

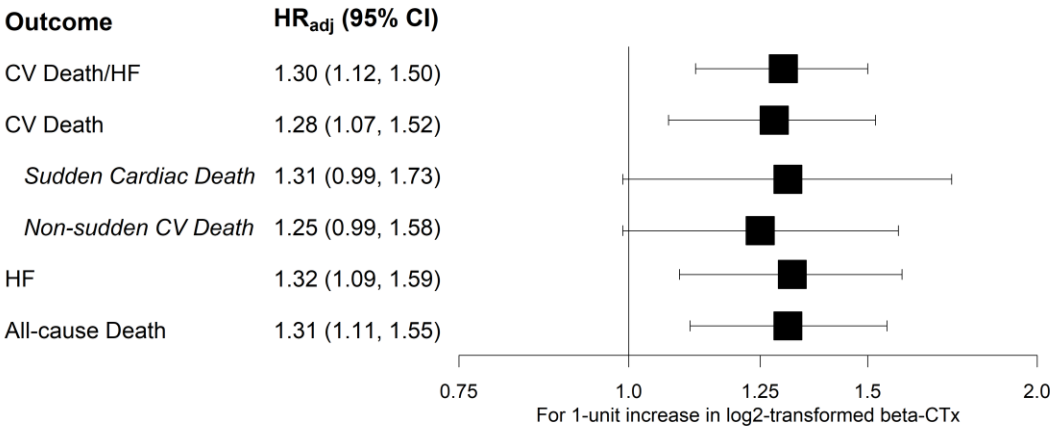



Figure S6. Adjusted hazard for 1-unit increase in log2-transformed beta-CTx serum concentrations and cardiovascular outcomes.