JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 80, NO. 10, 2022

Cathepsin S Levels and Survival Among Patients With Non-ST-Segment Elevation Acute Coronary Syndromes

Kimon Stamatelopoulos, MD,[a,b,*] Matthias Mueller-Hennessen, MD,[c,d,*] Georgios Georgiopoulos, MD,[a,b] Pedro Lopez-Ayala, MD,[e] Marco Sachse, MD,[f] Nikolaos I. Vlachogiannis, MD,[b] Kateryna Sopova, MD,[g,h] Dimitrios Delialis, MD,[a] Francesca Bonini, PhD,[f] Raphael Patras, MD,[a] Giorgia Ciliberti, MSc,[f] Mehrshad Vafaie, MD,[c] Moritz Biener, MD,[c] Jasper Boeddinghaus, MD,[e] Thomas Nestelberger, MD,[e] Luca Koechlin, MD,[e] Simon Tual-Chalot, PhD,[b] Ioannis Kanakakis, MD,[a] Aikaterini Gatsiou, PhD,[b] Hugo Katus, MD,[c,d] Ioakim Spyridopoulos, MD,[g,h] Christian Mueller, MD,[e] Evangelos Giannitsis, MD,[c,d,†] Konstantinos Stellos, MD[b,d,f,h,i,†]

ABSTRACT

BACKGROUND Patients with non-ST-segment elevation acute coronary syndromes (NSTE-ACS) are at high residual risk for long-term cardiovascular (CV) mortality. Cathepsin S (CTSS) is a lysosomal cysteine protease with elastolytic and collagenolytic activity that has been involved in atherosclerotic plaque rupture.

OBJECTIVES The purpose of this study was to determine the following: 1) the prognostic value of circulating CTSS measured at patient admission for long-term mortality in NSTE-ACS; and 2) its additive value over the GRACE (Global Registry of Acute Coronary Events) risk score.

METHODS This was a single-center cohort study, consecutively recruiting patients with adjudicated NSTE-ACS (n = 1,112) from the emergency department of an academic hospital. CTSS was measured in serum using enzyme-linked immunosorbent assay. All-cause mortality at 8 years was the primary endpoint. CV death was the secondary endpoint.

RESULTS In total, 367 (33.0%) deaths were recorded. CTSS was associated with increased risk of all-cause mortality (HR for highest vs lowest quarter of CTSS: 1.89; 95% CI: 1.34-2.66; $P < 0.001$) and CV death (HR: 2.58; 95% CI: 1.15-5.77; $P = 0.021$) after adjusting for traditional CV risk factors, high-sensitivity C-reactive protein, left ventricular ejection fraction, high-sensitivity troponin-T, revascularization and index diagnosis (unstable angina/ non-ST-segment elevation myocardial infarction). When CTSS was added to the GRACE score, it conferred significant discrimination and reclassification value for all-cause mortality (Delta Harrell's C: 0.03; 95% CI: 0.012-0.047; $P = 0.001$; and net reclassification improvement = 0.202; $P = 0.003$) and CV death (AUC: 0.056; 95% CI: 0.017-0.095; $P = 0.005$; and net reclassification improvement = 0.390; $P = 0.001$) even after additionally considering high-sensitivity troponin-T and left ventricular ejection fraction.

CONCLUSIONS Circulating CTSS is a predictor of long-term mortality and improves risk stratification of patients with NSTE-ACS over the GRACE score. (J Am Coll Cardiol 2022;80:998-1010) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Listen to this manuscript's audio summary by Editor-in-Chief Dr Valentin Fuster on www.jacc.org/journal/jacc.

From the [a]Department of Clinical Therapeutics, Alexandra Hospital, National and Kapodistrian University of Athens Medical School, Athens, Greece; [b]Biosciences Institute, Vascular Biology and Medicine Theme, Faculty of Medical Sciences, Newcastle University, Newcastle Upon Tyne, United Kingdom; [c]Department of Internal Medicine III, Cardiology, University Hospital Heidelberg, Heidelberg, Germany; [d]German Centre for Cardiovascular Research (DZHK), partner site Heidelberg/Mannheim, Mannheim, Germany; [e]Cardiovascular Research Institute Basel and University Hospital of Basel, Basel, Switzerland; [f]Department of Cardiovascular Research, European Center for Angioscience, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [g]Translational and Clinical Research Institute, Vascular Biology and Medicine Theme, Faculty of Medical Sciences, Newcastle University, Newcastle Upon Tyne, United Kingdom; [h]Department of Cardiology, Newcastle Hospitals NHS Foundation Trust, Newcastle Upon Tyne, United Kingdom; and the [i]Department of Cardiology, University Hospital Mannheim, Heidelberg University, Manheim, Germany. *Drs Stamatelopoulos and Mueller-Hennessen are co-first authors and contributed equally to this work. †Drs Giannitsis and Stellos are co-senior authors and contributed equally to this work.

ISSN 0735-1097 https://doi.org/10.1016/j.jacc.2022.05.055

Risk assessment scores and clinical prediction algorithms have been developed to help identify subjects who are at increased risk of incident coronary heart disease or to distinguish patients with acute coronary syndrome (ACS) who are at increased risk of adverse in-hospital or long-term cardiovascular (CV) outcomes.[1,2] Non-ST-segment elevation acute coronary syndromes (NSTE-ACS) comprise a heterogeneous disease population, and treatment decisions rely on risk stratification.[3-5] Long-term risk stratification and secondary prevention in NSTE-ACS patients is even more challenging, given that this population remains at a substantial residual risk despite optimal prevention strategies.[6] Interestingly, long-term morbidity and mortality in NSTE-ACS is similarly high to ST-segment elevation myocardial infarction (MI),[7] whereas stable coronary artery disease (CAD) patients with a history of previous ACS are at higher CV risk than those without an ACS history. Nevertheless, secondary prevention recommendations do not currently differentiate these 2 groups of stable CAD patients.[6,8] This substantially high late mortality in NSTE-ACS is underestimated[7] and indicates the need for new biomarkers to further refine long-term risk stratification and thus accurately identify NSTE-ACS patients who would or would not benefit from aggressive secondary prevention strategies.

SEE PAGE 1011

For this purpose, the GRACE (Global Registry of Acute Coronary Events) score, a well-established and validated risk score that is recommended for the acute management of NSTE-ACS,[3,4] has undergone rigorous validation for longer-term mortality in a series of cohort studies of post-ACS patients.[7,9] However, currently there is no definite recommendation for long-term risk stratification of these patients using the GRACE score or other clinical algorithms, highlighting a gap in evidence in this setting. To that end, blood-based biomarkers may be useful in the identification of residual risk for death or acute MI in patients with a previous ACS.[10-14] Cathepsin S (CTSS) is an extracellular matrix (ECM) degradation enzyme with both elastolytic and collagenolytic properties, which is also implicated in innate immune responses.[15,16] Animal studies support a central role of CTSS in atherogenesis and arterial tissue inflammation.[17,18] CTSS levels have also been found to be increased in human atherosclerotic arterial tissues,[19] as well as in the circulation of patients with CV disease and diabetes.[20] Interestingly, in 2 cohorts of elderly individuals, CTSS serum levels were associated with long-term mortality after a median follow-up of 12.6 and 7.9 years, respectively.[21] Thus, considering the strong preclinical and human evidence of the role of CTSS in atherosclerosis, we originally hypothesized that admission CTSS levels in ACS may reflect chronic matrix degradation processes involved in progressive atherosclerotic heart disease. Herein we sought to explore the long-term prognostic value of circulating CTSS and its additive and reclassification value over the GRACE score in NSTE-ACS patients.

ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome
CAD = coronary artery disease
CTSS = cathepsin S
CV = cardiovascular
ECM = extracellular matrix
hsCRP = high-sensitivity C-reactive protein
IDI = integrated discrimination index
MI = myocardial infarction
NRI = net reclassification improvement
NSTE-ACS = non-ST-segment elevation acute coronary syndromes

METHODS

POPULATION AND FOLLOW-UP. A total of 2,702 patients presenting to the emergency department of Heidelberg University Hospital, Germany, with a working diagnosis of ACS were consecutively recruited from June 2009 to April 2014 (**Figure 1**). Follow-up was performed by telephone contact and/or questionnaires sent by e-mail or land mail. Information on mortality events was further obtained by the local residents' registry. Despite the ongoing nature of this registry, an end-of-study date of follow-up was defined specifically for the current study (December 20, 2020). This date was selected to enable adequate and consistent monitoring for all patients and resulted in a median follow-up of 8.66 years with a maximum of 11.87 years. For consistency, all main analyses were performed for a fixed follow-up time of 8 years, and vital status was censored at this time point.

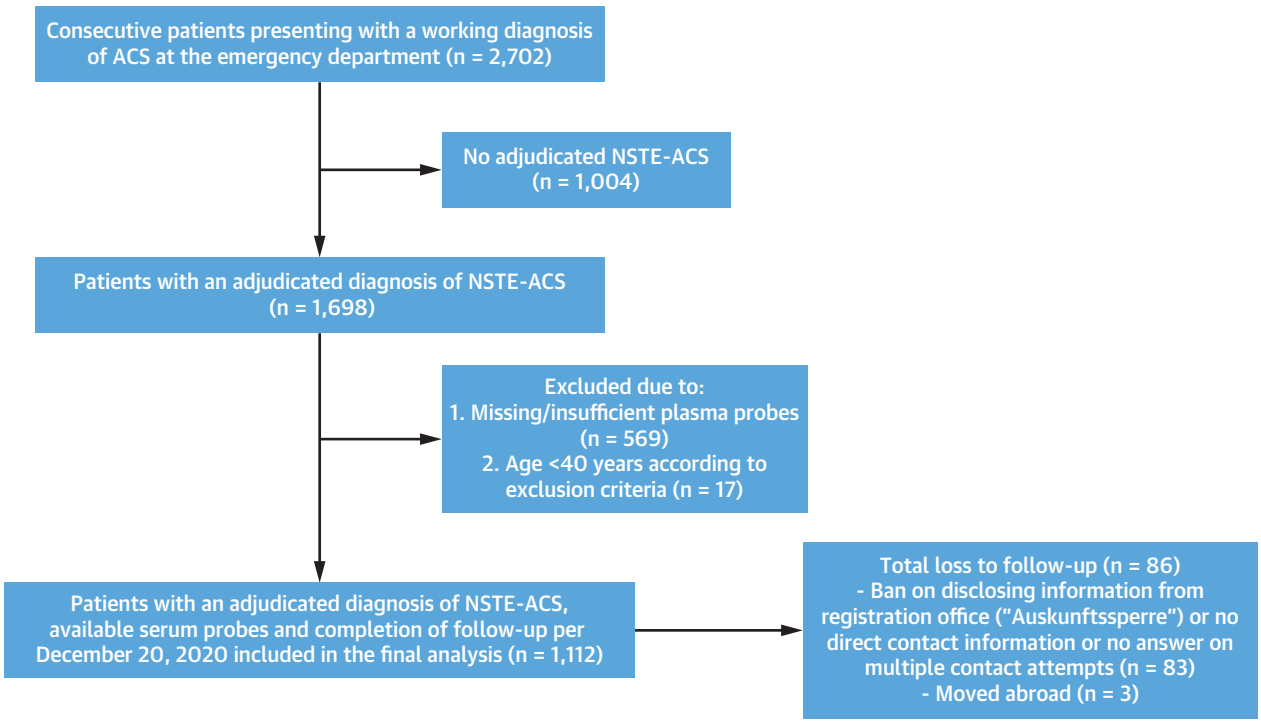
All-cause mortality was evaluated as the primary endpoint and CV death as the secondary endpoint of the study. The study was approved by the local institutional ethics committee. All patients provided written informed consent.

LABORATORY PARAMETERS AND BIOMARKER TESTING. The concentration of CTSS was measured in serum samples retrospectively with the help of a

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received March 18, 2022; revised manuscript received May 9, 2022, accepted May 31, 2022.

FIGURE 1 Flow Chart of the Study Population



Flow chart of the Heidelberg cohort. A total of 2,702 patients presenting to the emergency department of Heidelberg University Hospital with a working diagnosis of acute coronary syndrome (ACS) were consecutively recruited. Patients were retrospectively adjudicated, and analysis was restricted to a final diagnosis of non-ST-segment elevation acute coronary syndromes (NSTE-ACS) with available baseline blood samples (n = 1,112) for measurement of serum CTSS.

well-characterized enzyme immunoassay (interassay and intraassay variability 13.8% and 3.4%, respectively) (Human Total Cathepsin S DuoSet enzyme-linked immunosorbent assay DY1183, R&D Systems), as previously described.[21] Blood sampling was uniformly performed within 10 minutes from presentation at the emergency department and stored at −80 °C.

GRACE SCORE CALCULATION. GRACE score was calculated using the required variables on admission as previously described.[8,22]

STATISTICAL METHODS. Cox proportional hazards models were used to examine the association between CTSS levels and main outcomes of our study, and data were censored at the end-of-study date (December 20, 2020). Associations are presented as HR with 95% CIs. Nelson-Aalen curves were generated to depict the cumulative incidence of outcomes.

For all 3 outcomes, a nonlinear dose response association with continuous CTSS was observed. Therefore, the main analysis was based on continuous CTSS (dose-response curves) or quarters of CTSS. HRs are calculated between highest vs lowest quarter, per ascending quarter, or per comparison of 80th to 20th percentile of the baseline CTSS distribution (dose-response curves). An additional 2 sensitivity analyses were performed by selecting alternative sets of confounders (Supplemental Methods). Furthermore, we assessed the prognostic role and the additive predictive value of CTSS over the standard GRACE score for the study's endpoints in relevant multivariable Cox regression analyses and by calculating the following: 1) the likelihood ratio test; 2) the continuous net reclassification improvement (NRI); 3) the integrated discrimination improvement (IDI); and 4) the difference in the area under the curve (AUC) from time-dependent C-statistics (ie, Harrell's C) in the presence of censored data during the follow-up time.[23] We employed a resampling approach with 1,000 replicates to derive bootstrapped 95% CIs for NRI and IDI and increase robustness of the results. Statistical analysis was performed by the STATA package version 13.1 (StataCorp). All tests were 2-tailed. We deemed statistical significance at $P < 0.05$.

TABLE 1 Descriptive Characteristics of the Heidelberg Cohort by Quarters of Baseline Levels of CTSS

	Cathepsin S Quarters				
	1st Quarter (n = 278)	2nd Quarter (n = 278)	3rd Quarter (n = 278)	4th Quarter (n = 278)	*P* Value
Demographic characteristics					
Age, y	66.6 ± 12.3	68.8 ± 11.1	66.7 ± 10.9	68.6 ± 11.8	0.027
Male	189 (67.99)	193 (69.42)	190 (68.35)	173 (62.23)	0.267
T2DM	44 (15.83)	66 (23.74)	93 (33.45)	78 (28.06)	<0.001
Hyperlipidemia	172 (61.87)	178 (64.03)	179 (64.39)	159 (57.19)	0.276
Smoking	96 (36.78)	99 (36.40)	89 (33.33)	97 (36.47)	0.823
Arterial hypertension	216 (77.70)	223 (80.22)	220 (79.14)	226 (81.29)	0.75
History of CAD	126 (45.32)	134 (48.20)	147 (52.88)	122 (43.88)	0.154
DAPT	122 (43.88)	116 (41.73)	110 (39.57)	118 (42.45)	0.775
Statin	249 (91.54)	244 (90.37)	243 (90.33)	237 (88.43)	0.679
Beta-blocker	243 (89.34)	234 (86.67)	247 (91.48)	231(86.19)	0.187
Characteristics at presentation					
NSTEMI	192 (69.06)	197 (70.86)	206 (74.10)	208 (74.82)	0.382
SBP, mm Hg	151 ± 23.2	155 ± 23.2	149 ± 21.2	147 ± 26	0.003
GRACE >140	89 (32.01)	89 (32.01)	111 (39.93)	124 (44.60)	0.003
Killip class >1	12 (4.24)	12 (4.32)	21 (7.55)	25 (8.99)	0.008
LVEF <45%	88 (32.47)	82 (30.04)	105 (39.62)	116 (43.12)	0.005
SBP <110 mm Hg	10 (3.61)	5 (1.80)	7 (2.52)	21 (7.55)	0.002
Diseased coronary arteries	126 (52.07)	134 (53.82)	149 (61.32)	149 (60.32)	0.094
≥3 CAD vessels, n[a]	3 (1-3)	3 (2-3)	3 (2-3)	3 (1-3)	0.289
GRACE score[a]	128 (105-146)	129 (112-146)	134(112-152)	136 (113-159)	<0.001
hsTnT, ng/mL[a]	29 (7-206.9)	29 (9-155.3)	41.5(9-154)	65 (10-212)	0.086
hsCRP, mg/L[a]	2.3 (1.99-7.7)	2.9 (1.99-8.85)	3.45(1.99-11.45)	4.55 (1.99-19.05)	0.001
Glucose, mg/dL	127 ± 37.9	131 ± 44.9	139 ± 59.3	140 ± 66.7	0.013
eGFR, mL/min/1.73 m^2[a]	86.7 (78-96.3)	83.7 (76.3-91.5)	84.5 (73.6-94.5)	82.2 (70.6-91.5)	<0.001
CTSS, ng/mL[a]	4,705 (4,078-5,160)	6,258 (5,896-6,651)	8,037 (7,462-8,658)	11,810 (10,282-14,928)	<0.001
Patient management					
PTCA	160 (57.55)	148 (53.24)	158 (56.83)	144 (51.80)	0.457
CABG	11 (3.96)	11 (3.96)	20 (7.19)	18 (6.47)	0.199
Revascularization	169 (60.79)	159 (57.19)	178 (64.03)	162 (58.27)	0.36
Outcome					
Death at follow-up, incidence rate per 100 person-y	2.86 (2.18-3.74)	4.94 (4-6.08)	4.5 (3.6-5.6)	6.26 (5.15-7.6)	<0.001
CV death, incidence rate per 100 person-y	0.54 (0.29-1)	0.78 (0.46-1.33)	1.35 (0.9-2)	1.59 (1.09-2.35)	0.003
Days until death[a]	3,313 (2,951-3,966)	3,178 (2,397-3,601)	3,281 (2,733-3,575)	3,116 (2,060-3,534)	<0.001

Values are mean ± SD, n (%), or median (IQR). *P* value is derived from analysis of variance for continuous variables (or from the nonparametric Kruskal-Wallis test for non-normally distributed variables) and the chi-square test or the Jonckheere-Terpstra test for nominal variables. [a]*P* value is derived from nonparametric linear trend.

CABG = coronary artery bypass graft; CAD = coronary artery disease; CTSS = cathepsin S; CV = cardiovascular; DAPT = dual antiplatelet therapy; eGFR = estimated glomerular filtration rate; GRACE = Global Registry of Acute Coronary Events; hsCRP = high-sensitivity C-reactive protein; hsTnT = high-sensitivity troponin T; LVEF = left ventricular ejection fraction; MI = acute myocardial infarction; NSTEMI = non-ST-segment elevation myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty; SBP = systolic blood pressure; T2DM = type 2 diabetes mellitus.

RESULTS

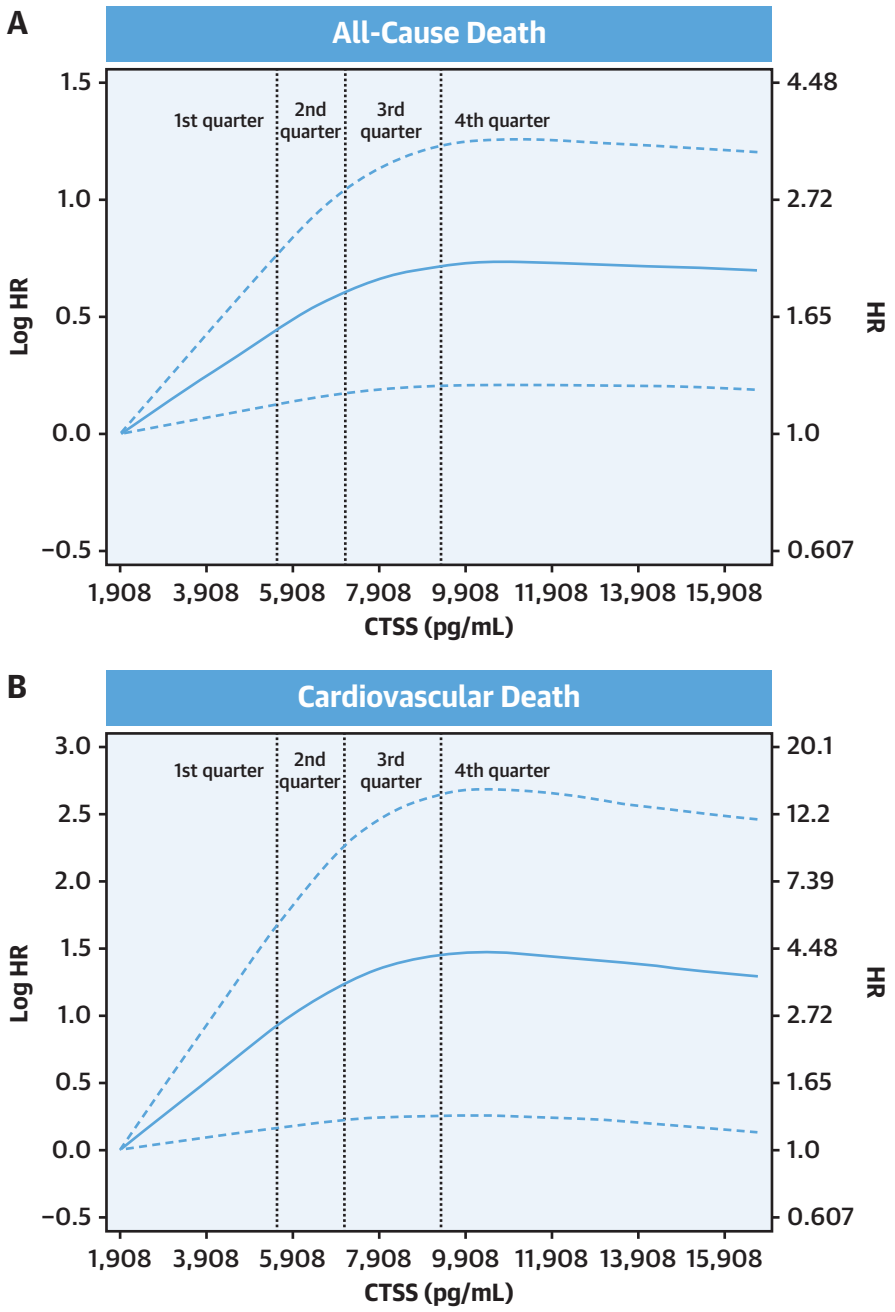
ASSOCIATION OF CTSS WITH CLINICAL RISK PROFILE OF NSTE-ACS PATIENTS. Descriptive characteristics of our study's population are outlined in Table 1. CTSS was associated with increased prevalence of diabetes mellitus, higher Killip class, GRACE score, high-sensitivity C-reactive protein (hsCRP) levels, and left ventricular systolic dysfunction and with lower systolic blood pressure and estimated glomerular filtration rate (eGFR) (Table 1).

Patients in higher quarters of CTSS showed higher incidence rate of all-cause mortality and CV mortality (Table 1).

INDEPENDENT ASSOCIATION OF CTSS WITH OUTCOMES. After a median follow-up of 8.66 years (25th-75th percentile: 4.88-9.79 years), 367 (33.0%) deaths were reported, of which 83 (7.46%) were of CV origin.

Dose-response curve analysis revealed a nonlinear association of continuous CTSS with all-cause death ($P = 0.004$ for nonlinearity) (Figure 2A) and CV death ($P = 0.009$ for nonlinearity) (Figure 2B).

FIGURE 2 **Smoothed Restricted Cubic Spline Plots of CTSS With the Study Endpoints**



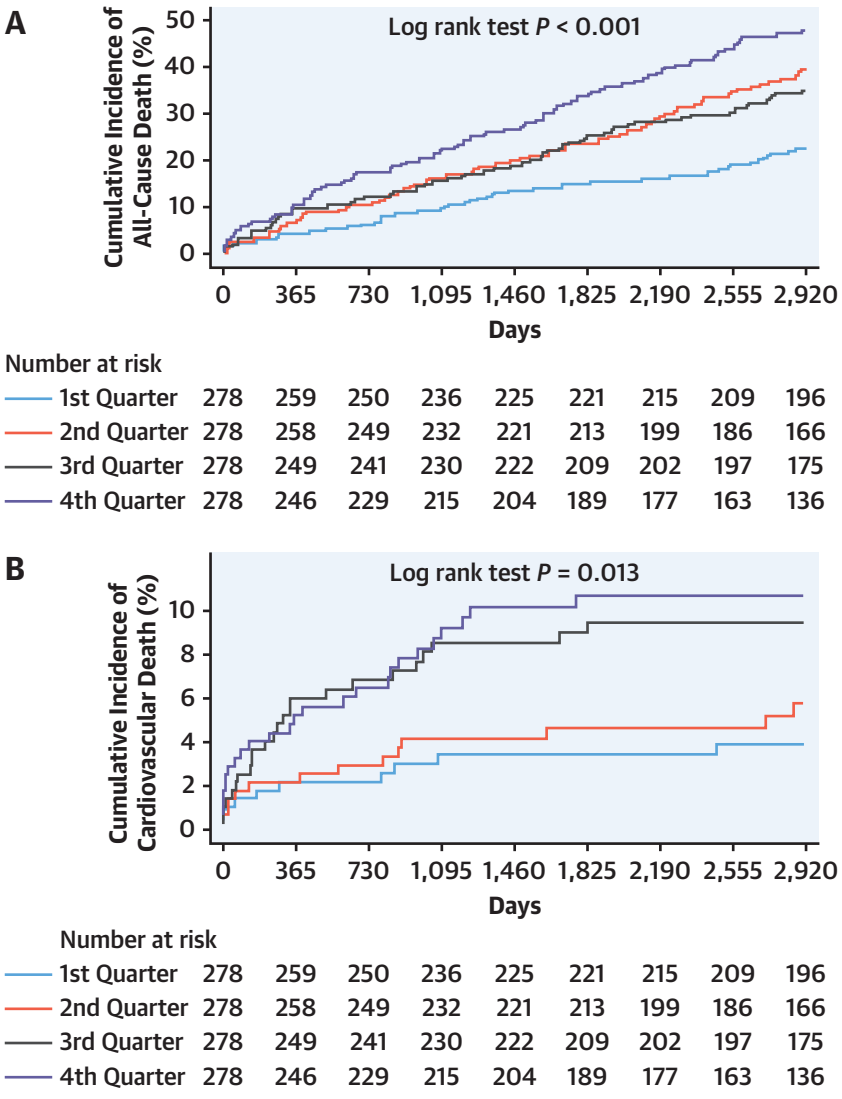
Three knots were fixed at the 10th, 50th, and 90th percentile of cathepsin S (CTSS) distribution for **(A)** all-cause death and **(B)** CV death. Quarters correspond to quarters of CTSS. Predicted HRs were estimated from a proportional hazards model adjusting for age >65 years, sex, diabetes mellitus, left ventricular ejection fraction, high-sensitivity troponin T, high-sensitivity C-reactive protein, revascularization, and index diagnosis of acute coronary syndrome. The **top and bottom dashed curves** represent the 95% CIs about the predicted HRs **(middle solid line)** in log scale **(left Y axis)** and the untransformed HRs **(right Y axis)**. **Vertical dashed lines** indicate the distribution of CTSS quarters. For visual clarity, the dose-response curves have been truncated after the 95th percentile of CTSS.

Overall, all-cause and CV death occurred more often in higher quarters of CTSS than the lowest quarter (**Figures 3A and 3B, Table 2**) (log-rank test $P <$ 0.05 for both). The association of higher baseline levels of CTSS with all-cause and CV death was also evinced when ascending quarters or inferomedian and supramedian cutoffs were used (**Figures 3C and 3D**). By Cox regression analysis, patients with CTSS in higher quarters presented increased risk for all-cause (HR: 1.89 for 4th vs lowest quarter of CTSS; 95% CI: 1.34-2.66; $P < 0.001$) and CV (HR: 2.58 for 4th vs lowest quarter of CTSS; 95% CI: 1.15-5.77; $P = 0.021$) mortality after controlling for age >65 years, sex, diabetes mellitus, left ventricular ejection fraction (LVEF), high-sensitivity cardiac troponin T (hsTnT), hsCRP, revascularization, and index diagnosis (**Table 2,** Supplemental Table 1). These associations remained significant after implementing ascending quarters of CTSS or continuous CTSS values (HR: 1.43 for 80th vs 20th centile in continuous CTSS; 95% CI: 1.11-1.83; $P = 0.005$ for all-cause mortality; and HR: 2.07 for 80th vs 20th centile in continuous CTSS; 95% CI: 1.16-3.70; $P = 0.014$ for CV mortality) (**Table 2**). We did not find significant interactions between CTSS and age or sex ($P > 0.05$ for all), suggesting no effect modification of CTSS with the prespecified events by these 2 variables. Of interest, when smaller time frames were used, we found that CTSS is clearly and independently related to all-cause death at 3 years, and this association seems to be mainly driven by CV death (Supplemental Table 2). At 8 years, CTSS retained its significant association with CV death and non-CV death (Supplemental Table 2).

SENSITIVITY ANALYSES. A priori–defined sensitivity analyses indicated that increased CTSS was associated with elevated risk for all-cause and CV death after additional adjustment for estimated glomerular filtration rate (all-cause death: HR: 1.72 for 4th vs lowest quarter of CTSS; 95% CI: 1.22-2.44; $P = 0.002$; and CV death: HR: 2.27 for 4th vs lowest quarter of CTSS; 95% CI: 1.01-5.13; $P = 0.048$) or Killip class (Supplemental Table 3), after replacing age >65 years with age as a continuous variable (Supplemental Table 3) or after excluding patients with diabetes mellitus (n = 281) (Supplemental Table 4). Considering the last patient enrolled into this study of our registry and the last contact for follow-up, a period of at least 5.5 years follow-up provided the highest possible integrity of complete prospective data (51 patients lost to follow-up; 4.5%). Thus, we reran the main analyses for a follow-up period of 5.5 years and found that all results from the analysis of 8 years follow-up were replicated (Supplemental Table 5). Similarly, we reran the same analysis for the full follow-up after exclusion of patients with incomplete follow-up information at 5.5 years and confirmed our original findings in the total population (Supplemental Table 6).

PROGNOSTIC AND RECLASSIFICATION VALUE OF CTSS. CTSS was associated with increased risk of all-cause (HR: 2.0 for highest vs lowest quarters of CTSS; 95% CI: 1.44-2.79; $P < 0.001$) and CV (HR: 2.55 for higher vs lowest quarters of CTSS; 95% CI: 1.23-5.28; $P = 0.012$) mortality after adjusting for the GRACE score (**Table 2**). When CTSS was added to the joint prognostic model, it conferred significant reclassification value for all-cause mortality (NRI = 0.202; 95% CI: 0.08-0.341; $P = 0.003$) and CV death (NRI = 0.390; 95% CI: 0.155-0.604; $P = 0.001$) (**Table 3**). Similarly, CTSS correctly discriminated patients who died (Delta AUC: 0.03; 95% CI: 0.012-0.047; $P = 0.001$ for all-cause death; AUC for CTSS on top of GRACE score: 0.66; 95% CI: 0.527-0.800; and Delta AUC: 0.056; 95% CI: 0.017-0.095; $P = 0.005$ for CV death) (**Table 3**). The incremental discriminative value of CTSS on top of the GRACE score, LVEF, and hsTnT for all-cause and CV mortality was further shown by significant IDI and likelihood ratio tests (**Table 3**). Because GRACE score yielded a relatively low discriminative value in our cohort, we further expanded our clinical core model to include a measure of left ventricular systolic function (LVEF) and of cardiac injury hsTnT. CTSS was associated with increased risk of all-cause mortality (HR: 1.9 for highest vs lowest quarters of CTSS; 95% CI: 1.35-2.66; $P < 0.001$) and CV mortality (HR: 2.48 for higher vs lowest quarters of CTSS; 95% CI: 1.15-5.31; $P = 0.02$) after controlling for the expanded clinical model (**Table 2**). The independent association of CTSS with all-cause death was driven by CV death at 3 years (Supplemental Table 2) but held true for both CV death and non-CV death at 8 years (Supplemental Table 2). Moreover, CTSS was associated with all-cause and CV death independently of the GRACE score, LVEF, and hsTnT across different time periods (ie, 5.5 years, where the minimum lost to follow-up was observed) (Supplemental Table 5) after excluding patients with missing follow-up information before 5.5 years (Supplemental Table 6) or diabetes (Supplemental Table 4). Importantly, CTSS retained its incremental reclassification and discrimination value over the combination of the GRACE score, LVEF, and hsTnT for both study survival outcomes (**Table 3**).

FIGURE 3 Nelson-Aalen Curves of CTSS Quarters With the Study Endpoints



Patients in the highest quarter vs lowest quarter of cathepsin S (CTSS) have the highest probability of all-cause death **(A)** and cardiovascular death **(B).** Patients in the highest quarter of CTSS vs lower quarters (vs the combination of second and third quarter vs lowest quarter) have the highest probability for all-cause mortality **(C),** and patients with CTSS levels above median have higher probabilities for cardiovascular death **(D)**.

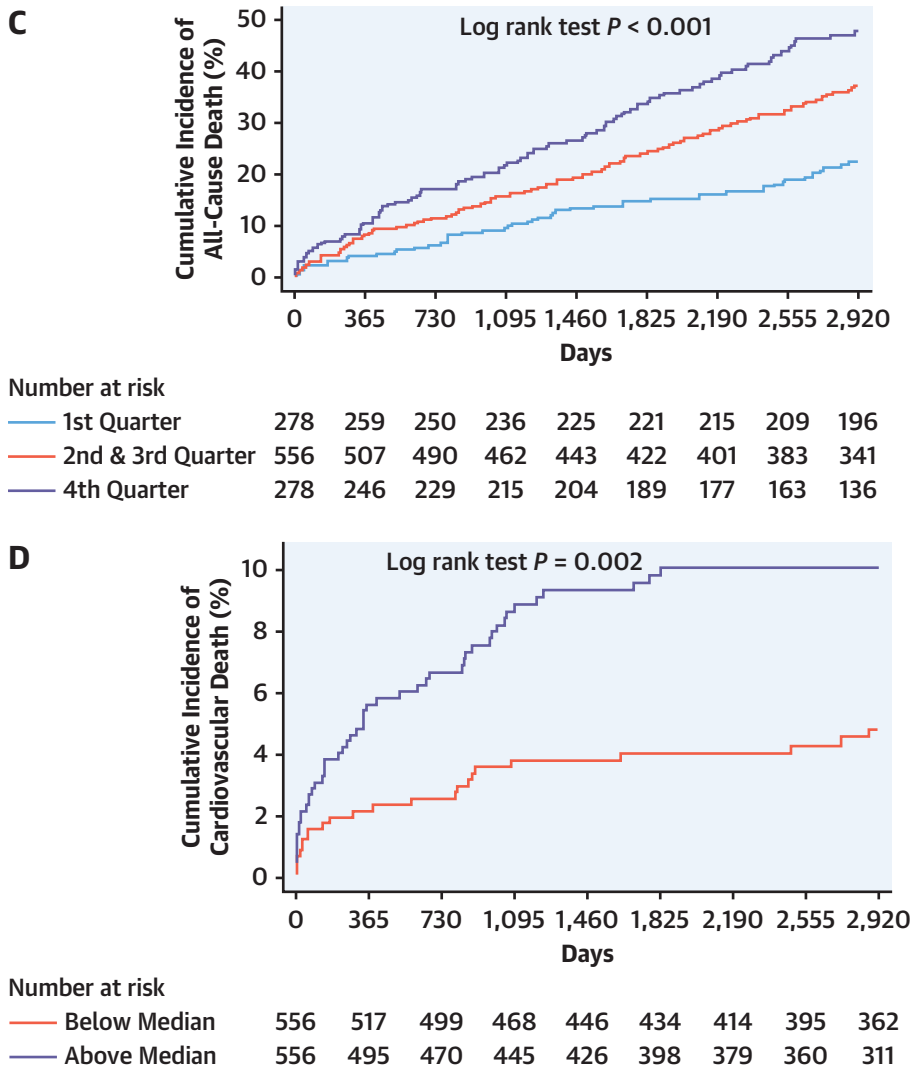
Continued on the next page

DISCUSSION

Our study provides evidence that a single measurement of serum CTSS at NSTE-ACS presentation is prognostic of long-term all-cause and CV mortality (**Central Illustration**). Importantly, CTSS serum levels improve the discrimination and reclassification value of the GRACE risk score, an established risk score widely recommended for treatment decisions of patients with NSTE-ACS and with good long-term prognostic ability.[7,9] The incremental prognostic value of CTSS levels over the GRACE score is retained even when validated biomarkers such as LVEF and hsTnT are additionally considered.

ACS patients have significant long-term residual risk despite application of aggressive treatment strategies.[6] The GRACE score has been well established as the most widely recommended score to risk-stratify

FIGURE 3 Continued



patients for in-hospital mortality and guide acute NSTE-ACS treatment.[7,9] However, a specific risk stratification strategy for longer-term prognosis has not been established; therefore, it is not uniformly recommended by international cardiology societies. Although the GRACE score had initially shown good performance for long-term prognosis in post-ACS patients,[7,9] its prognostic value was lower than expected in some validation cohorts.[24] Given that residual risk in ACS patients is partly attributed to under-recognized excessive inflammatory burden,[6] some limitations of the GRACE score and other risk stratification approaches may stem from not considering biomarkers reflecting inflammation or ECM degradation, 2 processes that are integrally involved in the regulation of myocardial remodeling post-MI,[25,26] as well as in atherosclerotic plaque composition and rupture.[27,28] Thus, further refinement of accurate risk stratification of NSTE-ACS patients by additionally assessing such biomarkers would possibly improve the accurate recognition of patients in need of aggressive secondary prevention and minimize unaddressed residual risk.

To that end, we found that CTSS, a lysosomal cysteine protease involved in both ECM degradation and inflammatory responses,[16] provided incremental reclassification and discriminative value over the GRACE score for the long-term prognosis of our NSTE-ACS population. Specifically, CTSS correctly discriminated and reclassified NSTE-ACS patients at

TABLE 2 Models for the Association of CTSS With All-Cause Mortality and Cardiovascular Mortality

	4th vs 1st Quarter		Per Ascending Quarter		Continuous CTSS[a]	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
All-cause mortality						
Univariable	2.16 (1.55-3.00)	<0.001	1.23 (1.12-1.36)	<0.001	1.57 (1.24-2.00)	<0.001
Adjusted for core model[b]	1.89 (1.34-2.66)	<0.001	1.18 (1.06-1.30)	0.002	1.43 (1.11-1.83)	0.005
Adjusted for the GRACE Score	2.00 (1.44-2.79)	<0.001	1.21 (1.09-1.33)	<0.001	1.48 (1.16-1.88)	0.002
Adjusted for GRACE score + LVEF + hsTnT	1.90 (1.35-2.66)	<0.001	1.17 (1.06-1.29)	0.002	1.43 (1.12-1.82)	0.004
CV death						
Univariable	2.76 (1.33-5.72)	0.006	1.41 (1.14-1.74)	0.002	2.35 (1.35-4.07)	0.003
Adjusted for core model[b]	2.58 (1.15-5.77)	0.021	1.34 (1.07-1.70)	0.011	2.07 (1.16-3.70)	0.014
Adjusted for the GRACE score	2.55 (1.23-5.28)	0.012	1.38 (1.11-1.71)	0.003	2.21 (1.27-3.85)	0.005
Adjusted for GRACE score + LVEF + hsTnT	2.48 (1.15-5.31)	0.02	1.33 (1.07-1.65)	0.01	2.13 (1.21-3.74)	0.009

[a]HR corresponds to comparison of the 80th and 20th percentile of continuous CTSS distribution using a restricted cubic spline transformation. [b]HRs are adjusted for the effect of age >65 y, sex, diabetes mellitus, hsTnT, hsCRP, revascularization, LVEF, and index diagnosis of ACS (NSTEMI/unstable angina).

Abbreviations as in Table 1.

actual high risk who were not correctly identified by the GRACE score. Interestingly, we found that both the reclassification and discrimination value of CTSS was higher for the prediction of CV events and particularly for CV death compared with all-cause mortality (NRI = 0.390 vs 0.202), suggesting a more specific prognostic ability for recurrent CV events. This observation was more pronounced at 3-year follow-up, with CTSS being more strongly associated with CV death than non-CV death. Indeed, accumulating evidence supports a central role of CTSS in chronic inflammation in atherosclerotic disease.[17-19,29-31] CTSS was one of the first ECM degradation enzymes to be detected at high levels at sites of elastic lamina breaks in atherosclerotic tissue.[19] ECM has been increasingly recognized as the orchestrator of the local inflammatory response, atherosclerotic plaque destabilization, and myocardial repair post-MI,[25,28] leading to increased interest in matrix-oriented therapeutics.[32] Of interest, CTSS-deficient mice showed impaired microvessel growth[18] and, most importantly, fewer plaque ruptures in the *Apoe*$^{-/-}$ mouse model.[33]

TABLE 3 Additive Discriminative and Reclassification Value of CTSS For All-Cause and CV Death

		Discrimination				Reclassification			
	Likelihood Ratio Test	C-Statistics		IDI		Continuous NRI			
Baseline Predictors	Chi-Square *P* Value	Delta AUC 95% CI	*P* Value	Overall 95% CI[a]	*P* Value	Events (%)	Nonevents (%)	Overall 95% CI[a]	*P* Value
All-cause death									
GRACE score	13.64 <0.001	0.03 (0.012 to 0.047)	0.001	1.57 (0.5 to 3.5)	<0.001	12.7 [47][b]	7.58 [56][c]	0.202 (0.08 to 0.341)	0.003
LVEF	12.87 <0.001	0.024 (0.008 to 0.039)	0.002	1.46 (0.4 to 3.3)	<0.001	12.02 [44]	8.28 [62]	0.203 (0.07 to 0.337)	0.003
hsTnT	14.11 <0.001	0.017 (0.063 to 0.027)	<0.001	1.53 (0.3 to 3.3)	<0.001	12.7 [47]	7.44 [55]	0.201 (0.077 to 0.335)	0.003
GRACE score + LVEF + hsTnT	9.67 0.002	0.008 (0.013 to 0.015)	0.019	1.04 (0.2 to 2.6)	0.002	12.02 [44]	8.14 [60.6]	0.201 (0.061 to 0.334)	0.003
CV death									
GRACE score	8.92 0.002	0.056 (0.017 to 0.095)	0.005	1.00 (0.1 to 2.7)	0.003	35.14 [29]	3.9 [40]	0.390 (0.155 to 0.604)	0.001
LVEF	8.11 0.004	0.043 (0.013 to 0.073)	0.005	0.96 (0.1 to 2.7)	0.008	35.22 [29]	4.58 [47]	0.397 (0.163 to 0.636)	0.001
hsTnT	8.79 0.003	0.026 (0.004 to 0.047)	0.017	0.86 (0.0 to 2.7)	0.037	35.14 [29]	3.8 [39]	0.389 (0.164 to 0.613)	0.001
GRACE score + LVEF + hsTnT	6.96 0.008	0.014 (−0.006 to 0.035)	0.175	0.82 (0.0 to 2.7)	0.039	35.22 [29]	4.46 [46]	0.396 (0.171 to 0.604)	0.001

[a]95% CI are derived from bootstrap with 1,000 replicates. [b]Brackets provide the net count of events assigned a higher risk. [c]Brackets provide the net count of nonevents assigned a lower risk

IDI = integrated discrimination improvement; NRI = net reclassification improvement; other abbreviations as in Table 1.

CENTRAL ILLUSTRATION **Summary of Our Hypothesis, Study Design, and Main Findings**

Background

Cathepsin S (CTSS) is a protease involved in:
- Extracellular matrix degradation
- Vascular inflammation and leukocyte migration
- Angiogenesis
- SMC invasion and migration
- Apoptosis

→ Atheroprogression and plaque rupture

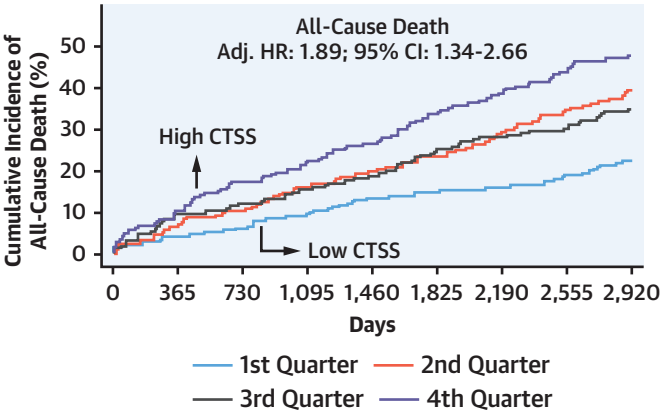
Hypothesis

Serum CTSS Levels May Identify Patients at High Risk for Death Post NSTE-ACS

Study Design

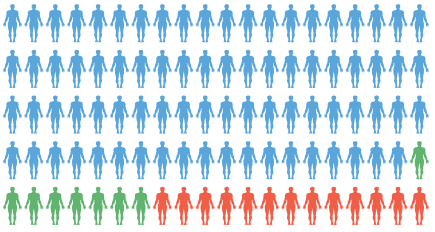
- 1,112 consecutive NSTE-ACS patients
- Single measurement of serum CTSS at admission
- 8 years follow-up
- Primary endpoint: all-cause death
- Secondary endpoint: CV death

CTSS Is Associated With Long-Term Residual Risk in Patients Post NSTE-ACS



CTSS Improves Risk Stratification of Patients Post NSTE-ACS

CTSS correctly reclassifies over GRACE Score
12.7% of patients who died into higher risk and
7.6% of patients who survived into lower risk



Stamatelopoulos K, et al. J Am Coll Cardiol. 2022;80(10):998–1010.

Cathepsin S (CTSS) is a protease involved in extracellular matrix remodeling and inflammation. Herein we examined the prognostic and reclassification value of circulating CTSS for long-term mortality in non–ST-segment elevation acute coronary syndromes (NSTE-ACS) (n = 1,112). All-cause mortality at 8 years was the primary study endpoint. CTSS was associated with increased risk of all-cause mortality (adjusted HR: 1.89; 95% CI: 1.34-2.66 for highest vs lowest CTSS quarter) after adjusting for traditional cardiovascular (CV) risk factors, high-sensitivity C-reactive protein, left ventricular ejection fraction, high-sensitivity troponin-T, revascularization, and index diagnosis (unstable angina/non–ST-segment elevation myocardial infarction). When CTSS was added to the GRACE (Global Registry of Acute Coronary Events) score, it conferred significant reclassification value for all-cause mortality (net reclassification improvement = 0.202). SMC = smooth muscle cell.

In line with the experimental evidence, CTSS expression and activity have been found to be increased in unstable carotid plaques in humans,[34] while circulating CTSS has been found increased in patients with multiple CV disease risk factors including obesity,[35] diabetes mellitus,[20] and systemic inflammation (as assessed by hsCRP and interleukin [IL]-6 levels).[36] Similarly, increased CTSS levels have been detected both in the circulation[20] and at the vessel wall of patients with atherosclerosis,[19] indicating that increased CTSS is linked to adverse proatherosclerotic processes and vascular inflammation. In support of this notion, we found that high serum CTSS levels are associated with an adverse clinical risk profile at the acute phase of an NSTE-ACS. Specifically, patients with increased CTSS presented with an increased risk profile, including higher prevalence of diabetes mellitus; lower systolic blood pressure and eGFR; left ventricular systolic dysfunction; as well as higher hsCRP, GRACE score, and Killip class. These observations, combined with the independent association of CTSS with increased mortality in NSTE-ACS, support a clinical role of CTSS in these patients.

Although, to the best of our knowledge, this is the first evidence assessing and demonstrating the prognostic value of CTSS in ACS, the CLARICOR (Effect of Clarithromycin on Mortality and Morbidity in Patients With Ischemic Heart Disease) study failed to show a similar significant association in stable CAD patients.[37] This discordance may be attributed to different CV risk profile characteristics between stable CAD patients with and without a previous ACS.[6]

Accumulating evidence indicates that stable CAD patients with a history of prior ACS have persistently higher long-term risk of adverse CV outcomes compared with stable CAD patients without a previous ACS despite optimal treatment.[6] Although this differentiation is the result of a combination of factors, sustained increased inflammatory load is considered a major contributor.[6] Given that in the CLARICOR study, both derivation and validation cohorts comprised a mixed population of stable CAD patients and predominantly of stable CAD patients without a previous ACS (68%), the prognostic role of CTSS in stable CAD patients with a previous ACS remains yet to be clarified. Our findings showing that CTSS predicts long-term cardiac adverse events in NSTE-ACS patients support this concept. Interestingly, we found that CTSS was also associated with noncardiac mortality. This finding may be explained by previous evidence associating CTSS with noncardiac diseases such as cancer, Alzheimer's disease, chronic obstructive pulmonary disease, and chronic kidney disease.[38] Whether CTSS could also serve as a biomarker of risk for the incidence or progression of these noncardiac diseases merits investigation.

Some therapeutic implications arise from our findings when combined with current literature. The antiatherosclerotic effects of CTSS targeting in animal models,[17,29,33] its role as a regulator of ECM degradation and inflammatory response,[15,17,31] as well as the overall safe profile of CTSS-targeting monoclonal antibodies in early phases of clinical trials (eg, NCT02679014) warrant further investigation of the clinical value of CTSS-targeting therapies in NSTE-ACS patients. Indeed, targeting an atherosclerosis-specific inflammatory pathway by canakinumab, an IL-1b-neutralizing monoclonal antibody, led to significant reduction of systemic inflammation[39] and major adverse CV events in ACS patients.[40] Interestingly, despite the beneficial prognostic effect of canakinumab in ACS patients, there was substantial residual risk even in those patients treated with the drug.[39] This was attributed to high levels of other inflammatory molecules such as those of IL-6 and -18.[39] Thus, assessment of different inflammatory pathways may confer additive prognostic effects, and individually targeting them might incrementally improve CV outcomes. To that end, developing a clinical assay for CTSS measurement to identify patients eligible for anti-CTSS immunotherapy would be the first step toward the evaluation of CTSS as a possible therapeutic target in NSTE-ACS. Whether other cathepsins also confer an incremental value of GRACE risk score in the prediction of survival in patients with NSTE-ACS remains unknown. Future studies are warranted to evaluate the prognostic value of other cathepsins in comparison to CTSS in these patients

STUDY LIMITATIONS. First, although the outcomes were prospectively recorded, the hypothesis of this study was retrospectively evaluated in this cohort. Serial measurements of CTSS during the course of ACS or at later time points were not available. Although from the stand point of pathophysiology, serial measurements would provide deeper insight to a causal association between CTSS and prognosis, assessing the utility of initial levels of a biomarker at ACS presentation may still confer long-term prognostic data and provides clinically relevant information to the optimal short- and long-term management of ACS patients.[41-46] As described in the previous text, there is robust experimental evidence supporting a causal link between CTSS and atherosclerosis. Whether CTSS may be used as a biomarker to assist clinical decision-making needs to be confirmed in future studies investigating the prognostic value of the temporal assessment of CTSS levels in serial time points post-NSTE-ACS as well as the effect of guideline-suggested medical therapy on CTSS levels. Overall, our findings encourage the further evaluation of CTSS as a clinically relevant biomarker. In addition, although AUC values for CTSS were relatively low, we found that CTSS could correctly reclassify risk in a substantial proportion of patients reaching >35% for CV death over a robust clinical core model including GRACE score, hsTnT, and LVEF. Furthermore, CTSS improved well-established discrimination (IDI) and calibration indexes (likelihood ratio)[47-49] over the same model. Given that C-statistics interpreted alone may severely underestimate the prognostic value of a biomarker,[50-52] it is a common practice in contemporary biomarker statistics to implement such approaches of multiple statistical indexes to address this limitation. Furthermore, natriuretic peptide measurements, which could further allow contextualization of our findings with contemporary guidelines, were unavailable in our cohort. Finally, although we performed bootstrap resampling for reclassification analyses, which is considered a form of internal validation, the main study findings should be also externally validated in other NSTE-ACS cohorts. This is especially important, because our results point toward a meaningful and clinically useful prognostic value of serum CTSS measurement for long-term mortality. Considering that our findings suggest that measuring serum CTSS may provide complementary nonoverlapping prognostic information over validated conventional predictors and the GRACE risk

score, future studies are warranted to confirm the prognostic value of CTSS as a residual risk biomarker that may be useful for the long-term risk stratification post NSTE-ACS.

CONCLUSIONS

Herein, we report that increased circulating levels of CTSS in NSTE-ACS patients, measured during the acute phase of the syndrome, are associated with an adverse risk profile at presentation and confer independent long-term prognostic value. Importantly, combined with the GRACE score, a reliable prognostic tool in ACS, CTSS improved risk stratification in terms of reclassification and discrimination. Effective secondary prevention in NSTE-ACS patients remains a major challenge in cardiology because of increased long-term residual risk despite optimal treatment in these patients. New therapies targeting CV-specific inflammatory pathways are effective in mitigating this risk. Given that CTSS exerts proinflammatory atherosclerotic properties, the novel, hypothesis-generating findings of this study point toward future research to externally validate circulating CTSS as a prognostic and therapeutic biomarker in NSTE-ACS.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This research was funded by the German Research Foundation DFG (SFB834 project number 75732319) and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 759248) (to Dr Stellos). Dr Sopova was supported with a scholarship from the German Heart Foundation (Deutsche Herzstiftung). The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Konstantinos Stellos, Department of Cardiovascular Research, European Center for Angioscience, Heidelberg University, Ludolf-Krehl-Straße 13-17, D-68167 Mannheim, Germany. E-mail: konstantinos.stellos@medma.uni-heidelberg.de. OR Dr Kimon Stamatelopoulos, Vascular Laboratory, Department of Clinical Therapeutics, Alexandra Hospital, Medical School, National and Kapodistrian University of Athens, P.O. Box 11528, 80 Vas. Sofias Str, Athens, Greece. E-mail: kstamatel@med.uoa.gr.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Serum levels of the matrix degradation enzyme CTSS are related to all-cause and CV mortality in patients with NSTE-ACS, and have reclassification and discriminative value over the GRACE risk score.

TRANSLATIONAL OUTLOOK: Future studies should investigate clinical applications of CTSS as a therapeutic target in patients with NSTE-ACS.

REFERENCES

1. Rossello X, Dorresteijn JA, Janssen A, et al. Risk prediction tools in cardiovascular disease prevention: a report from the ESC Prevention of CVD Programme led by the European Association of Preventive Cardiology (EAPC) in collaboration with the Acute Cardiovascular Care Association (ACCA) and the Association of Cardiovascular Nursing and Allied Professions (ACNAP). *Eur J Prev Cardiol*. 2019;26:1534–1544.

2. Bawamia B, Mehran R, Qiu W, Kunadian V. Risk scores in acute coronary syndrome and percutaneous coronary intervention: a review. *Am Heart J*. 2013;165:441–450.

3. Roffi M, Patrono C, Collet JP, et al. 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting Without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2016;37:267–315.

4. Amsterdam EA, Wenger NK, Brindis RG, et al. 2014 AHA/ACC guideline for the management of patients with non-ST-elevation acute coronary syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014;64:e139–e228.

5. Wright RS, Anderson JL, Adams CD, et al. 2011 ACCF/AHA focused update of the Guidelines for the Management of Patients with Unstable Angina/Non-ST-Elevation Myocardial Infarction (updating the 2007 guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines developed in collaboration with the American College of Emergency Physicians, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *J Am Coll Cardiol*. 2011;57:1920–1959.

6. Gallone G, Baldetti L, Pagnesi M, et al. Medical therapy for long-term prevention of atherothrombosis following an acute coronary syndrome: JACC state-of-the-art review. *J Am Coll Cardiol*. 2018;72:2886–2903.

7. Fox KA, Carruthers KF, Dunbar DR, et al. Underestimated and under-recognized: the late consequences of acute coronary syndrome (GRACE UK-Belgian Study). *Eur Heart J*. 2010;31:2755–2764.

8. Fox KA, Dabbous OH, Goldberg RJ, et al. Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observational study (GRACE). *BMJ*. 2006;333:1091.

9. Fox KA, Fitzgerald G, Puymirat E, et al. Should patients with acute coronary disease be stratified for management according to their risk? Derivation, external validation and outcomes using the updated GRACE risk score. *BMJ Open*. 2014;4:e004425.

10. Ng LL, Sandhu JK, Narayan H, et al. Pro-substance P for evaluation of risk in acute myocardial infarction. *J Am Coll Cardiol*. 2014;64:1698–1707.

11. Widera C, Pencina MJ, Meisner A, et al. Adjustment of the GRACE score by growth differentiation factor 15 enables a more accurate appreciation of risk in non-ST-elevation acute coronary syndrome. *Eur Heart J*. 2012;33:1095–1104.

12. Morrow DA, Sabatine MS, Brennan ML, et al. Concurrent evaluation of novel cardiac biomarkers in acute coronary syndrome: myeloperoxidase and soluble CD40 ligand and the risk of recurrent

ischaemic events in TACTICS-TIMI 18. *Eur Heart J*. 2008;29:1096–1102.

13. Hagström E, James SK, Bertilsson M, et al. Growth differentiation factor-15 level predicts major bleeding and cardiovascular events in patients with acute coronary syndromes: results from the PLATO study. *Eur Heart J*. 2016;37:1325–1333.

14. Wallentin L, Lindholm D, Siegbahn A, et al. Biomarkers in relation to the effects of ticagrelor in comparison with clopidogrel in non-ST-elevation acute coronary syndrome patients managed with or without in-hospital revascularization: a substudy from the Prospective Randomized Platelet Inhibition and Patient Outcomes (PLATO) trial. *Circulation*. 2014;129:293–303.

15. Honey K, Rudensky AY. Lysosomal cysteine proteases regulate antigen presentation. *Nat Rev Immunol*. 2003;3:472–482.

16. Liu CL, Guo J, Zhang X, Sukhova GK, Libby P, Shi GP. Cysteine protease cathepsins in cardiovascular disease: from basic research to clinical trials. *Nat Rev Cardiol*. 2018;15:351–370.

17. Sukhova GK, Zhang Y, Pan JH, et al. Deficiency of cathepsin S reduces atherosclerosis in LDL receptor-deficient mice. *J Clin Invest*. 2003;111:897–906.

18. Shi GP, Sukhova GK, Kuzuya M, et al. Deficiency of the cysteine protease cathepsin S impairs microvessel growth. *Circ Res*. 2003;92:493–500.

19. Sukhova GK, Shi GP, Simon DI, Chapman HA, Libby P. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest*. 1998;102:576–583.

20. Liu J, Ma L, Yang J, et al. Increased serum cathepsin S in patients with atherosclerosis and diabetes. *Atherosclerosis*. 2006;186:411–419.

21. Jobs E, Ingelsson E, Risérus U, et al. Association between serum cathepsin S and mortality in older adults. *JAMA*. 2011;306:1113.

22. Granger CB, Goldberg RJ, Dabbous O, et al. Predictors of hospital mortality in the global registry of acute coronary events. *Arch Intern Med*. 2003;163:2345–2353.

23. Newson RB. Comparing the predictive powers of survival models using Harrell's C or Somers' D. *The Stata Journal*. 2010;10:339–358.

24. Palmerini T, Genereux P, Caixeta A, et al. A new score for risk stratification of patients with acute coronary syndromes undergoing percutaneous coronary intervention: the ACUITY-PCI (Acute Catheterization and Urgent Intervention Triage Strategy-Percutaneous Coronary Intervention) risk score. *J Am Coll Cardiol Intv*. 2012;5:1108–1116.

25. Frangogiannis NG. The extracellular matrix in myocardial injury, repair, and remodeling. *J Clin Invest*. 2017;127:1600–1612.

26. Seropian IM, Toldo S, Van Tassell BW, Abbate A. Anti-inflammatory strategies for ventricular remodeling following ST-segment elevation acute myocardial infarction. *J Am Coll Cardiol*. 2014;63:1593–1603.

27. Ross R. Atherosclerosis-an inflammatory disease. *N Engl J Med*. 1999;340:115–126.

28. Shah PK, Galis ZS. Matrix metalloproteinase hypothesis of plaque rupture: players keep piling up but questions remain. *Circulation*. 2001;104:1878–1880.

29. Samokhin AO, Lythgo PA, Gauthier JY, Percival MD, Brömme D. Pharmacological inhibition of cathepsin S decreases atherosclerotic lesions in Apoe-/- mice. *J Cardiovasc Pharmacol*. 2010;56:98–105.

30. Stellos K, Gatsiou A, Stamatelopoulos K, et al. Adenosine-to-inosine RNA editing controls cathepsin S expression in atherosclerosis by enabling HuR-mediated post-transcriptional regulation. *Nat Med*. 2016;22:1140–1150.

31. Shi GP, Villadangos JA, Dranoff G, et al. Cathepsin S required for normal MHC class II peptide loading and germinal center development. *Immunity*. 1999;10:197–206.

32. Traverse JH, Henry TD, Dib N, et al. First-in-man study of a cardiac extracellular matrix hydrogel in early and late myocardial infarction patients. *J Am Coll Cardiol Basic Trans Science*. 2019;4:659–669.

33. Rodgers KJ, Watkins DJ, Miller AL, et al. Destabilizing role of cathepsin S in murine atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*. 2006;26:851–856.

34. Abd-Elrahman I, Meir K, Kosuge H, et al. Characterizing cathepsin activity and macrophage subtypes in excised human carotid plaques. *Stroke*. 2016;47:1101–1108.

35. Taleb S, Cancello R, Poitou C, et al. Weight loss reduces adipose tissue cathepsin S and its circulating levels in morbidly obese women. *J Clin Endocrinol Metab*. 2006;91:1042–1047.

36. Jobs E, Risérus U, Ingelsson E, et al. Serum cathepsin S is associated with serum C-reactive protein and interleukin-6 independently of obesity in elderly men. *J Clin Endocrinol Metab*. 2010;95:4460–4464.

37. Wuopio J, Hilden J, Bring C, et al. Cathepsin B and S as markers for cardiovascular risk and all-cause mortality in patients with stable coronary heart disease during 10 years: a CLARICOR trial sub-study. *Atherosclerosis*. 2018;278:97–102.

38. De Pasquale V, Moles A, Pavone LM. Cathepsins in the pathophysiology of mucopolysaccharidoses: new perspectives for therapy. *Cells*. 2020;9(4):979.

39. Ridker PM, MacFadyen JG, Thuren T, Libby P. Residual inflammatory risk associated with interleukin-18 and interleukin-6 after successful interleukin-1β inhibition with canakinumab: further rationale for the development of targeted anti-cytokine therapies for the treatment of atherothrombosis. *Eur Heart J*. 2020;41:2153–2163.

40. Ridker PM, Everett BM, Thuren T, et al. Anti-inflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377:1119–1131.

41. Stamatelopoulos K, Mueller-Hennessen M, Georgiopoulos G, et al. Amyloid-β (1-40) and mortality in patients with non-ST-segment elevation acute coronary syndrome: a cohort study. *Ann Intern Med*. 2018;168:855–865.

42. Monteiro S, Monteiro P, Gonçalves F, Freitas M, Providência LA. Hyperglycaemia at admission in acute coronary syndrome patients: prognostic value in diabetics and non-diabetics. *Eur J Cardiovasc Prev Rehabil*. 2010;17:155–159.

43. Collet JP, Thiele H, Barbato E, et al. 2020 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J*. 2021;42:1289–1367.

44. Omland T, Ueland T, Jansson AM, et al. Circulating osteoprotegerin levels and long-term prognosis in patients with acute coronary syndromes. *J Am Coll Cardiol*. 2008;51:627–633.

45. Gravning J, Smedsrud MK, Omland T, et al. Sensitive troponin assays and N-terminal pro-B-type natriuretic peptide in acute coronary syndrome: prediction of significant coronary lesions and long-term prognosis. *Am Heart J*. 2013;165:716–724.

46. Kvisvik B, Mørkrid L, Røsjø H, et al. High-sensitivity troponin T vs I in acute coronary syndrome: prediction of significant coronary lesions and long-term prognosis. *Clin Chem*. 2017;63:552–562.

47. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med*. 2008;27:157–172. discussion 207–212.

48. Pencina MJ, D'Agostino RB, Pencina KM, Janssens AC, Greenland P. Interpreting incremental value of markers added to risk prediction models. *Am J Epidemiol*. 2012;176:473–481.

49. Pencina MJ, D'Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med*. 2011;30:11–21.

50. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. *Circulation*. 2007;115:928–935.

51. Cook NR, Buring JE, Ridker PM. The effect of including C-reactive protein in cardiovascular risk prediction models for women. *Ann Intern Med*. 2006;145:21–29.

52. Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med*. 2006;355:2631–2639.

KEY WORDS cathepsin S, GRACE, NSTE-ACS, reclassification, residual risk, risk stratification

APPENDIX For an expanded Methods section and supplemental tables, please see the online version of this paper.