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Soluble Urokinase-Type Plasminogen Activator Receptor Improves Risk Prediction in Patients With Chronic Heart Failure

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ABSTRACT

OBJECTIVES This study investigated the predictive value of soluble urokinase-type plasminogen activator receptor (suPAR) in patients with chronic heart failure (CHF).

BACKGROUND SuPAR originates from proteolytic cleavage of the membrane-bound receptor from activated immune and endothelial cells and reflects the level of immune activation. As inflammation plays a crucial role in the complex pathophysiology of CHF, we hypothesized that suPAR might be a suitable prognostic biomarker in patients with CHF.

METHODS SuPAR levels were determined in 319 patients with CHF admitted to our outpatient department for heart failure and in a second cohort consisting of 346 patients with CHF, for validation.

RESULTS During a median follow-up time of 3.2 years, 119 patients (37.3%) died. SuPAR was a strong predictor of mortality with a crude hazard ratio (HR) per increase of 1 SD (HR per 1 SD) of 1.96 (95% confidence interval [CI]: 1.63 to 2.35; $p < 0.001$) in univariate analysis and remained significant after comprehensive multivariate adjustment with an adjusted HR per 1 SD of 1.38 (95% CI: 1.04 to 1.83; $p = 0.026$). SuPAR added prognostic value beyond the multivariate model indicated by improvements in C-statistics (area under the curve: 0.72 vs 0.74, respectively; $p = 0.02$), the category-free net reclassification index (24.9%; $p = 0.032$), and the integrated discrimination improvement (0.011; $p = 0.05$). Validation in the second cohort yielded consistent results.

CONCLUSIONS SuPAR is a strong and independent predictor of mortality in patients with CHF, potentially suitable to refine risk assessment in this vulnerable group of patients. Our results emphasize the impact of immune activation on survival in patients with CHF. (J Am Coll Cardiol HF 2017;5:268–77) © 2017 by the American College of Cardiology Foundation.

Chronic heart failure (CHF) is a highly prevalent clinical syndrome with almost epidemic proportions due to aging of the society, constant increase of predisposing risk factors, and improved survival rates after cardiovascular events (1). The concept of biomarkers for diagnosis and prognosis is attractive, and introduction of natriuretic peptides has dramatically changed the standard of care in patients with CHF (2). Thus, exploration of novel biomarkers, which provide incremental prognostic information, may help to further improve management of patients and adapt therapies to the individual needs of patients. Systemic levels of soluble urokinase-type plasminogen activator receptor

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(suPAR) originates from proteolytic cleavage and release of the membrane-bound receptor from a variety of cell types (3). Elevated levels of suPAR reflect the level of immune activation in different pathological conditions ranging from infectious diseases to chronic inflammatory disorders to systemic inflammatory response syndrome and are associated with a poor prognosis in various patient populations (4,5). Beyond suPAR's role as marker of inflammatory processes, recent studies show a close correlation between suPAR levels and impaired renal function, and elevated concentrations predispose for a more rapid decline in glomerular filtration rate (GFR) (6,7). Both of the aforementioned pathophysiological conditions, inflammation and renal impairment, play a crucial role in genesis and progression of CHF, and biomarkers from both of these spectra are associated with impaired survival in patients with CHF (8,9).

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Soluble ST2 (sST2) is part of the interleukin (IL)-33/ST2 system, which regulates cardiac remodeling after myocardial infarction and protects myocardium from hypertrophy and fibrosis (10,11). Excess sST2 antagonizes beneficial effects of IL-33/ST2 signaling in the heart and promotes myocardial dysfunction and fibrosis (10–12). Similar to suPAR, sST2 has been implicated in a variety of acute and chronic clinical conditions, such as cardiovascular, renal, pulmonary, and malignant diseases. (13). In patients with both acute and chronic heart failure, sST2 is an established prognostic biomarker, with a Class II recommendation for additive risk stratification by American College of Cardiology Foundation/American Heart Association (14–16). On the basis of the aforementioned background and characteristics similar to sST2, we hypothesized that suPAR might be a suitable prognostic biomarker in patients with CHF. To test this hypothesis, we investigated the predictive value of suPAR in 319 patients with a specific focus on gain in prognostic information beyond that assessable with conventional CHF-related risk factors. Furthermore, we aimed to directly compare the prognostic value of suPAR with that of sST2. For validation of results, we analyzed suPAR in a second cohort consisting of 343 patients with advanced CHF. Finally, as there is an overwhelming increase in biomarker studies in the field of CHF with varying quality and questionable clinical utility, the evaluation of novel biomarkers needs to follow stringent rules. We therefore aimed to evaluate our results according to recommendations of Ahmad et al. (17) for the qualification of suPAR as a potential prognostic biomarker in CHF.

METHODS

STUDY POPULATION. Participants in both study cohorts provided written informed consent. The study protocols complied with the Declaration of Helsinki, and both studies were approved by the ethics committee of the Medical University of Vienna.

Derivation cohort. Patients included in this prospective observational study were consecutively enrolled at the outpatient department for heart failure at the Medical University of Vienna between January 2008 and July 2013. Inclusion criteria were defined as clinical signs of CHF (New York Heart Association [NYHA] functional class ≥2) and either levels of N-terminal pro B-type natriuretic peptide (NT-proBNP) of >500 pg/ml or a left ventricular ejection fraction of <40%. Exclusion criteria were presence of a non-cardiovascular comorbidity reducing life expectancy to <2 years, chronic inflammatory diseases, <18 years of age, and refusal to provide informed consent. Baseline characteristics were assessed by professional health care employees using a standardized patient questionnaire.

Validation cohort. For validation of results, suPAR levels were determined in a second cohort consisting of 346 patients with advanced CHF enrolled between July 2003 and September 2004. Patients were included in this cohort according to the following criteria: hospitalization due to cardiac decompensation, NYHA functional class III or IV at the time of admission, and a left ventricular ejection fraction of <40%.

STUDY ENDPOINTS AND FOLLOW-UP. Primary study endpoints were defined as all-cause mortality and cardiovascular mortality. Data for outcomes were assessed by scanning the national death registry (Statistik Austria) and crosschecking the local electronic clinical database (18). Death certificates of decedents were obtained to classify as cardiovascular and non-cardiovascular causes of death, using International Classification of Disease-10th Revision criteria.

LABORATORY MEASUREMENTS. Blood samples were obtained by venipuncture at time of enrollment. Standard laboratory measurements were determined from fresh samples. NT-proBNP levels were assessed by electrochemiluminescence (Elecsys 2010 unit, Roche Diagnostics, Basel, Switzerland). EDTA plasma samples were centrifuged at 3,000 rpm for 20 min at 50°C and stored at −80°C for further analyses. Blood sample tubes were stored at 4°C after blood draw for a maximum of 2 h before centrifugation. Plasma

ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

CART = classification and regression tree analysis

CHF = chronic heart failure

CRP = C-reactive protein

eGFR = estimated glomerular filtration rate

IDI = integrated discrimination increment

NRI = net reclassification index

NT-proBNP = N-terminal pro B-type natriuretic peptide

NYHA = New York Heart Association

suPAR = soluble urokinase-type plasminogen activator receptor

concentrations of suPAR were determined from initially thawed samples, using the suPARnostic standard enzyme-linked immunosorbent assay (ViroGates, Birkerød, Denmark). The assay measures all forms of circulating suPAR, including full-length and cleaved forms. The lowest limit of detection was 0.1 ng/ml. Intra-assay and interassay coefficients of variation determined in samples with a suPAR concentration ranging from 2.3 to 7.2 ng/ml were 1.3% to 4.7% and 1.7% to 5.1%, respectively. No sample had a variation >10%. sST2 was analyzed using U.S. Food and Drug Administration-approved Presage ST2 assay (Critical Diagnostics, San Diego, California) and determined after a second freeze-thaw cycle. Intra-assay and interassay coefficients of variation measured in samples with an sST2 concentration ranging from 16.9 to 159.1 ng/ml were 2.4% to 6.5% and 4.8% to 9.1%, respectively.

STATISTICAL ANALYSIS. Continuous variables are presented as medians and interquartile ranges (IQR) and categorical data as counts and percentages. Comparisons between groups were performed using a chi-square test, Mann-Whitney *U* test, and Kruskal-Wallis test, as appropriate. Spearman's rank correlation coefficient was used to estimate correlations between continuous variables. Univariate and multivariate Cox proportional hazard models were applied to assess the effect of suPAR on survival of patients. Model 1 was adjusted for age and sex. Model 2 was additionally adjusted for conventional CHF risk factors including NYHA functional classification, left ventricular ejection fraction, cause of CHF, systolic blood pressure, estimated GFR (eGFR; calculated using the Modification of Diet in Renal Disease formula), type 2 diabetes, atrial fibrillation (AF), hypertension, chronic obstructive pulmonary disease, and active smoking. Model 3 was additionally adjusted for NT-proBNP, and C-reactive protein (CRP). Continuous data were logarithmically transformed on a natural logarithm scale before being entered into the model and are hazard ratio (HR) per 1 increase of a log-transformed SD (HR per 1 SD). Kaplan-Meier plots were constructed to compare survival in groups according to suPAR tertiles. Harrell's C-statistic was applied to evaluate the discriminatory power to predict mortality. Category-free net reclassification improvement and integrated discrimination increment (IDI) were calculated to estimate an improvement in individual risk prediction for the addition of suPAR to established risk factors. Classification and regression tree (CART) analysis was applied to determine optimal cutoff values for suPAR and NT-proBNP in order to classify groups at high or low risk. A p value of <0.05 (2-tailed) was considered statistically significant. All statistical analyses were performed using STATA 12 software (StataCorp, College Station, Texas) and SPSS software version 20.0 (SPSS, IBM, Armonk, New York).

RESULTS

DISTRIBUTION OF suPAR AND BASELINE CHARACTERISTICS. suPAR levels were available in 319 patients, with a median concentration of 3.69 ng/ml (IQR: 2.84 to 5.26 ng/ml). Detailed baseline characteristics according to tertiles of suPAR are presented in **Table 1**. Briefly, the median age was 64.6 years (IQR: 56.7 to 71.1 years), 259 patients (81.2%) were male, 149 patients (46.7%) had ischemic causes of CHF, 144 patients (44.1%) were in NYHA functional class III or IV, and the median NT-proBNP concentration was 1,215 pg/ml (IQR: 477 to 2,632 pg/ml). Higher suPAR levels were significantly associated with NYHA functional class and common CHF-related comorbidities including type 2 diabetes, hypertension, AF, and chronic obstructive pulmonary hypertension (**Table 1**). Furthermore, suPAR showed significant correlations with eGFR ($r = -0.62$), NT-proBNP ($r = 0.51$), CRP ($r = 0.40$), sST2 ($r = 0.38$) and age ($r = 0.36$; all correlations with a p value <0.001) (Online Figure 1).

suPAR AND RISK PREDICTION. Patients were followed for a median of 3.2 years (IQR: 2.0 to 4.9 years). During this period, 119 patients (37.3%) died, including 82 patients (25.7%) who died due to cardiovascular causes. Cox proportional hazard models were applied to assess the impact of suPAR on mortality (**Table 2**, Online Tables 1 to 3). SuPAR was a strong predictor of mortality with a crude HR per 1 SD of 1.96 (95% confidence interval [CI]: 1.63 to 2.35; $p < 0.001$) in univariate Cox regression. Corresponding Kaplan-Meier curves showing survival according to tertiles of suPAR and an optimal cutoff of suPAR using CART analysis are plotted in **Figure 1** (log-rank test: $p < 0.001$). The optimal cutoff to predict all-cause mortality was 4.4 ng/ml, and 115 patients (36.1%) had values above this threshold (Online Table 4). Several multivariate models were used to analyze whether the prognostic value of suPAR was independently associated with mortality (**Table 2**). SuPAR remained a significant predictor of mortality even after full adjustment for potential confounders with an adjusted HR per 1 SD of 1.38 (95% CI: 1.04 to 1.83; $p = 0.026$) (**Table 2**, Model 3). Besides suPAR, only age, male sex, NYHA functional class, and NT-proBNP concentration were independently associated with mortality in this model. In direct comparison to sST2, suPAR had a higher crude HR per 1 SD

TABLE 1 Baseline Characteristics (N = 319)

	1st Tertile (n = 107) suPAR 2.6 ng/ml (IQR: 2.3–2.9 ng/ml)	2nd Tertile (n = 106) suPAR 3.7 ng/ml (IQR: 3.4–4.1 ng/ml)	3rd Tertile (n = 106) suPAR 6.1 ng/ml (IQR: 5.3–7.3 ng/ml)	p Value
Clinical characteristics				
Age, yrs	59.5 (51.8–66.6)	66.3 (56.7–71.1)	66.6 (61.6–73.9)	**<0.001**
Males, %	88 (82.2)	82 (77.4)	89 (84.0)	0.751
Body mass index, kg/m^2	28.4 (25.5–30.3)	29.1 (25.1–32.2)	27.8 (24.8–31.2)	0.418
Ischemic CHF, %	41 (38.3)	53 (50)	55 (51.9)	**0.047**
NYHA functional class, %				**<0.001**
II	77 (71.9)	62 (58.5)	36 (34.0)	
III	28 (26.2)	44 (41.5)	65 (61.3)	
IV	2 (1.9)	0 (0)	5 (4.7)	
LVEF, %				0.332
Mild	32 (29.9)	35 (33.0)	33 (31.1)	
Moderate	47 (43.9)	40 (37.7)	26 (24.5)	
Severe	28 (26.2)	31 (29.3)	47 (44.4)	
Systolic blood pressure, mm Hg	127 (117–143)	125 (110–140)	122 (110–140)	0.169
Heart rate, beats/min	67 (60–75)	70 (62–80)	71 (65–80)	0.084
Active smoker, %	23 (21.5)	28 (26.4)	18 (17)	0.427
Comorbidities				
Previous MI, %	38 (35.5)	49 (46.2)	47 (44.3)	0.192
Atrial fibrillation, %	31 (29.0)	49 (46.2)	56 (52.8)	**<0.001**
Hypertension, %	74 (69.2)	81 (76.4)	93 (87.7)	**0.001**
Diabetes mellitus, %	24 (22.4)	42 (39.6)	54 (50.9)	**<0.001**
COPD, %	18 (16.8)	24 (22.6)	39 (36.8)	**0.001**
Laboratory parameters				
NT-proBNP, pg/ml	509 (278–1,311)	1,136 (584–1,820)	2,816 (1,137–4,636)	**<0.001**
eGFR (MDRD), ml/min	75.5 (65.8–86.5)	62.3 (49.1–80.5)	43.8 (33.3–57.1)	**<0.001**
Cholesterol, mg/dl	187 (165–220)	178 (153–208)	165 (139–203)	**0.004**
CRP, mg/dl	2 (1.0–3.9)	2.9 (1.2–6.4)	5.2 (2.9–9.8)	**<0.001**
Cardiac medication				
Beta-blockers, %	106 (99.1)	104 (98.1)	100 (94.3)	**0.038**
ACE inhibitors/ARBs, %	107 (100.0)	106 (100.0)	106 (100.0)	1.000
Aldosterone antagonist, %	64 (59.8)	74 (69.8)	72 (67.9)	0.212
Diuretic agents, %	34 (31.8)	52 (49.1)	79 (74.5)	**<0.001**

Values are median (interquartile range) or n (%). Baseline characteristics for patients with CHF of the derivation cohort according to tertiles of soluble urokinase-type plasminogen activator receptor. Comparisons between groups were performed using a chi-square test and Kruskal-Wallis test as appropriate. The p values in **bold** indicate a value <0.05.

ACE = angiotensin converting enzyme; ADP = adenosine diphosphate receptor; ARB = angiotensin receptor blockers; CHF = chronic heart failure; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; eGFR = estimated glomerular filtration rate; LVEF = left ventricular ejection fraction; MI = myocardial infarction; NT-proBNP = N-terminal pro B-type natriuretic peptide; NYHA = New York Heart Association functional classification; suPAR = soluble urokinase-type plasminogen activator receptor.

(1.96 [95% CI: 1.63 to 2.35] vs. 1.63 [95% CI: 1.38 to 1.94], respectively, both with p < 0.001) (Table 2, Online Table 5). In addition, whereas suPAR remained significantly associated with mortality in the fully adjusted Model 3, sST2 lost its association with mortality and cardiovascular mortality (Online Table 5).

COMBINED ASSESSMENT OF suPAR AND NT-proBNP. To analyze, whether suPAR provided prognostic information to that determinable with NT-proBNP, we divided patients into 4 groups by combined strata of high or low suPAR and high or low NT-proBNP levels (Figure 2). The cutoff for high and low for both biomarker was determined using CART analysis (suPAR: ≤/>4.4 ng/ml, NT-proBNP: ≤/>2,133 pg/ml). The risk associated with high levels of suPAR were regardless of the NT-proBNP category (p for interaction: 0.424) (Figure 2A, see HRs). Compared with the group with low suPAR and low NT-proBNP, the group with high suPAR and high NT-proBNP had an HR of 6.57 (95% CI: 4.27 to 10.11; p < 0.001).

DISCRIMINATION AND RECLASSIFICATION. The area under the curve (AUC; Harrell's C-statistic) to predict mortality increased from 0.69 (95% CI: 0.64 to 0.72) for NT-proBNP alone to 0.72 (95% CI: 0.68 to 0.76, p for comparison: 0.02) for NT-proBNP (Table 3) suPAR and was confirmed by significant

TABLE 2 Survival Analysis

	All-Cause Mortality		Cardiovascular Mortality	
	HR (95% CI) per 1 SD	p Value	HR (95% CI) per 1 SD	p Value
Derivation cohort				
Univariate	1.96 (1.63–2.35)	**<0.001**	1.94 (1.56–2.41)	**<0.001**
Multivariate				
Model 1*	1.83 (1.52–2.22)	**<0.001**	1.80 (1.43–2.27)	**<0.001**
Model 2†	1.62 (1.26–2.08)	**<0.001**	1.72 (1.27–2.32)	**<0.001**
Model 3‡	1.40 (1.06–1.86)	**0.019**	1.36 (0.96–1.93)	0.083
Validation cohort				
Univariate	1.68 (1.45–1.95)	**<0.001**	1.55 (1.30–1.84)	**<0.001**
Multivariate				
Model 1*	1.60 (1.37–1.88)	**<0.001**	1.45 (1.20–1.74)	**<0.001**
Model 2†	1.56 (1.30–1.87)	**<0.001**	1.39 (1.12–1.73)	**0.003**
Model 3‡	1.45 (1.19–1.77)	**<0.001**	1.26 (1.01–1.60)	0.053

Univariate and multivariate Cox proportional hazard models were applied to assess the effect of soluble urokinase-type plasminogen activator receptor on survival of patients. The p values in **bold** indicate a value of <0.05. *Model 1 was adjusted for age and sex. †Model 2 was adjusted for Model 1 plus New York Heart Association functional classification, left ventricular ejection fraction, cause of chronic heart failure, systolic blood pressure, estimated glomerular filtration rate (MDRD), diabetes, atrial fibrillation, hypertension, chronic obstructive pulmonary disease, and active smoking. ‡Model 3 was adjusted for Model 2 plus N-terminal pro B-type natriuretic peptide, C-reactive protein, and soluble ST2 (derivation cohort).

CI = confidence interval; HR = hazard ratio.

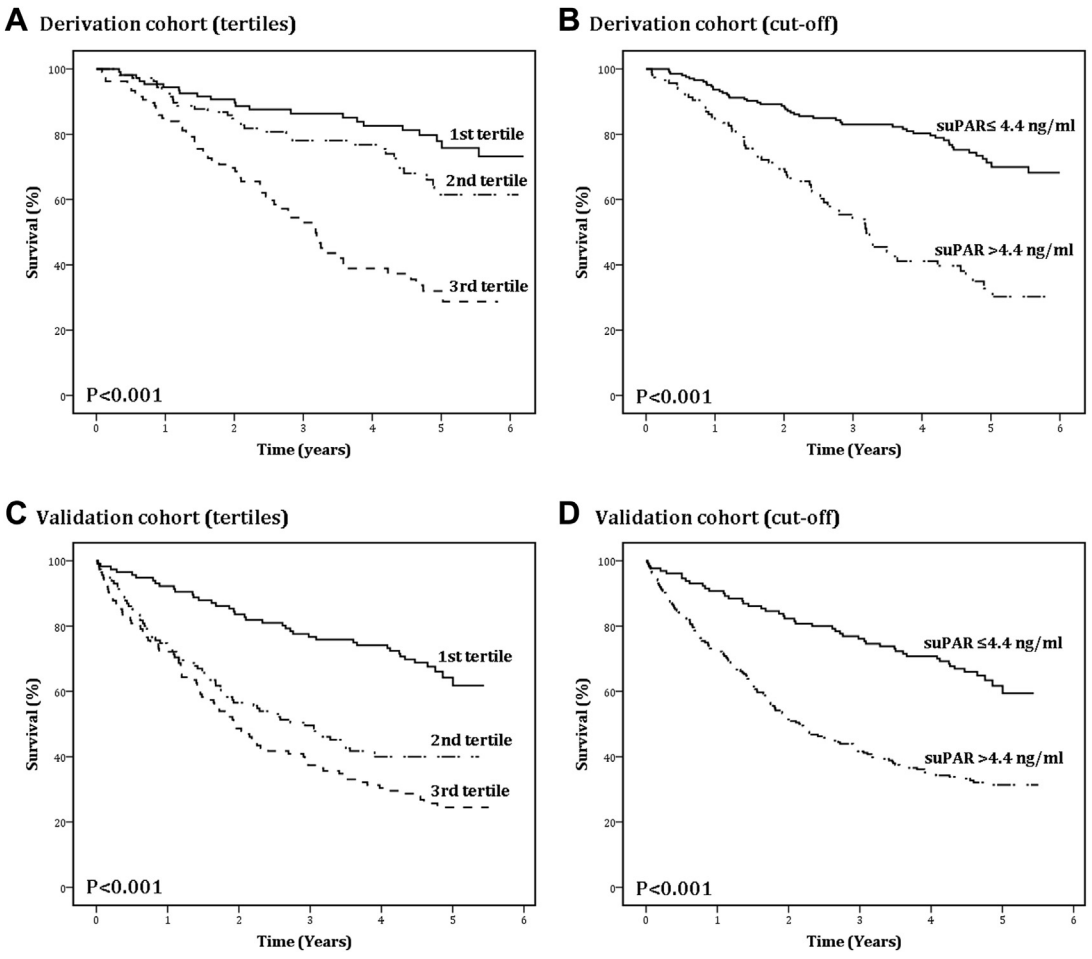
improvements in net reclassification index (NRI; HR: 32.9% [95% CI: 21.3 to 44.5], p = 0.006) and IDI (HR: 0.022 [95% CI: 0.014 to 0.028], p = 0.008) (Table 3). When we used all variables independently associated with mortality in multivariate Cox regression (age, male sex, NYHA functional class, and NT-proBNP), the AUC was 0.72 (95% CI: 0.68 to 0.75). Adding suPAR to this model resulted in a significant improvement in the AUC to 0.74 (95% CI: 0.70 to 0.78; p for comparison: 0.02) (Table 3). Again, suPAR showed significant improvements in NRI of 24.9% (95% CI: 13.3 to 36.5%, p = 0.032) and IDI of 0.011 (95% CI: 0.006 to 0.017, p = 0.05) when added to the multivariate model (Table 3).

VALIDATION OF RESULTS. To verify our initial findings, we measured plasma levels of suPAR in a second cohort of 346 patients with advanced CHF. Detailed baseline characteristics according to tertiles of suPAR and a comparison between cohorts are shown in Online Tables 6 and 7. In the validation cohort, 195 patients (56.4%) died during a median follow-up time of 3.6 years (IQR: 1.2 to 6.3 years). In Cox regression analysis, we achieved results similar to those of the derivation cohort, showing that suPAR was significantly and independently associated with mortality with a crude HR per 1 SD of 1.68 (95% CI: 1.45 to 1.95; p < 0.001) and an adjusted HR per 1 SD of 1.45 (95% CI: 1.19 to 1.77; p < 0.001) in the fully adjusted multivariate model (Table 2). Corresponding survival curves according to tertiles of suPAR and according to the pre-specified cutoff determined in the derivation cohort are shown in Figure 1. Of note, CART analysis showed only a marginal difference in cutoff values for mortality for suPAR compared to those for the derivation cohort (4.2 ng/ml vs. 4.4 ng/ml, respectively). Finally, we were able to confirm the incremental prognostic value beyond that over NT-proBNP levels (NRI: 54.9% [95% CI: 43.9 to 65.8%]; p < 0.001; IDI: 0.060 [95% CI: 0.047 to 0.073]; p < 0.001) and the multivariate model (NRI: 35.8% [95% CI: 24.8 to 46.7%]; p = 0.001; IDI: 0.022 [95% CI: 0.013 to 0.030]; p = 0.01) (Table 3). Compared with the group with low suPAR and low NT-proBNP levels, the group with high suPAR and high NT-proBNP levels had an HR of 4.49 (95% CI: 2.85 to 7.07) (Figure 2B).

DISCUSSION

In the present study, we analyzed the prognostic value of suPAR plasma levels in patients with CHF. We provide novel evidence that suPAR is a strong and independent predictor of mortality, which adds incremental prognostic information beyond that assessable with NT-proBNP assay and conventional cardiovascular risk factors, suggesting suPAR is a potential candidate to improve risk prediction in this group of patients. In addition, suPAR was a superior prognostic marker in direct comparison to sST2, an established guideline biomarker recommended for additional risk stratification in CHF. So far a few studies have linked suPAR to cardiovascular diseases. Two independent studies demonstrated an increased risk of cardiovascular events and mortality in the general population independent of potential confounders (4,19). Further studies showed an independent association of suPAR with subclinical vascular organ damage indicated by associations with pulse wave velocity, carotid atherosclerotic plaques and urine albumin/creatinine ratio (20,21). Beyond testing for patients in stable condition, suPAR is also a useful predictor of mortality and recurrent cardiovascular events in patients with acute coronary syndrome (22). Lyngbæk et al. (22) achieved interesting results by comparing serial measurements of suPAR with CRP in patients with ST-segment elevation myocardial infarction. Although CRP levels were significantly elevated during the first 24 h after symptom onset, suPAR levels remained relatively unchanged. In addition, neither baseline nor peak CRP levels were associated with mortality or recurrent myocardial infarction, whereas suPAR was an independent predictor of future events. In our study, suPAR was significantly associated with markers of CHF severity

FIGURE 1 Survival Curves

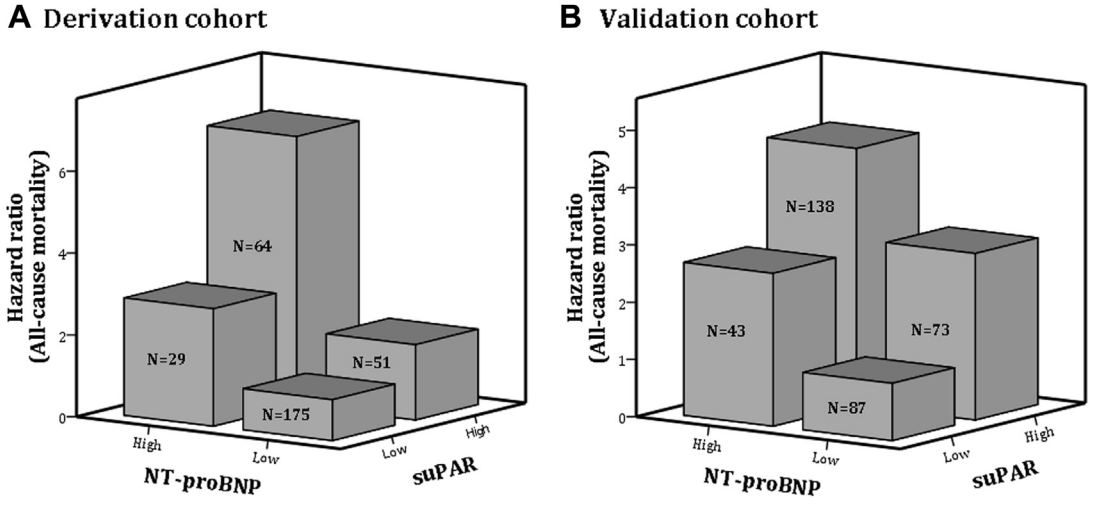


Kaplan-Meier estimates of the derivation **(A, B)** and validation cohort **(C, D)** according to tertiles **(A, C)** and an optimal cutoff **(B, D)** of soluble urokinase-type plasminogen activator receptor (suPAR).

including NT-proBNP levels and NYHA functional class. In addition, patients in the validation cohort, which consisted of patients hospitalized due to CHF, had significantly higher plasma levels than the derivation cohort. As such, higher suPAR levels seem directly related to disease stage and may increase during decompensation of CHF. However, the advanced age of patients and a higher proportion of concomitant comorbidities, which are all related to higher suPAR levels, may be important confounders. These differences may also be responsible for the slightly divergent HRs and the differences in the C-statistics between the cohorts. In addition, suPAR showed a more robust association with mortality from all causes than cardiovascular mortality in both cohorts. As such, suPAR seems to be a good, albeit integrative, prognostic biomarker, which is directly related to the clinical condition of patients and incorporates a variety of comorbidities and organ dysfunctions. Initial evidence for an association of suPAR implicated in occurrence of CHF derive from a study by Borne et al. (23) demonstrating an independent association with incident heart failure in the general population even after adjustment for clinical variables and biomarkers, including cystatin C, NT-proBNP, and CRP. We extended the knowledge derived from this study of suPAR and new-onset of CHF by demonstrating that suPAR is an independent prognosticator in patients with existing CHF.

Following the impact of suPAR on survival of patients with CHF, the possible mechanisms underlying

FIGURE 2 Risk in Combined Strata



Relative risk of mortality according to combined strata of soluble urokinase-type plasminogen activator receptor (suPAR) and N-terminal pro B-type natriuretic peptide (NT-proBNP) cutoffs for the derivation **(A)** and validation cohort **(B)**.

this observation and the question of whether suPAR is a marker of, or contributor to, progressive heart failure needs to be discussed. Chronic inflammation is an important pathophysiological aspect of CHF. Proinflammatory cytokines such as tumor necrosis factor-α induce left ventricular dysfunction by promoting myocardial remodeling through hypertrophy of cardiomyocytes, induction of myocardial fibrosis, and progressive loss of myocytes through apoptosis (9). Proinflammatory cytokines stimulate release of suPAR from activated neutrophils, monocytes, and endothelial cell by proteolytic cleavage from the cell surface into the plasma (3,24,25). SuPAR itself maintains the inflammatory process by acting as chemotactic agent contributing to recruitment of immune cells to the sites of acute inflammation (25). In our

TABLE 3 Discrimination and Reclassification

	Derivation Cohort		Validation Cohort	
	AUC (95% CI)	**p Value**	**AUC (95% CI)**	**p Value**
Harrell's C-Statistic				
NT-proBNP	0.69 (0.64–0.72)		0.65 (0.61–0.68)	
NT-proBNP plus suPAR	0.72 (0.68–0.76)	for comparison: **0.04**	0.68 (0.64–0.71)	For comparison: 0.17
Multivariate model	0.72 (0.68–0.75)		0.68 (0.64–0.71)	
Multivariate model plus suPAR	0.74 (0.70–0.78)	for comparison: **0.02**	0.70 (0.66–0.74)	For comparison: 0.32
	Improvement (95% CI)		**Improvement (95% CI)**	
Net reclassification index				
suPAR in addition to NT-proBNP	32.9% (21.3%–44.5%)	**0.006**	54.9% (43.9%–65.8%)	**<0.001**
suPAR in addition to multivariate model	24.9% (13.3%–36.5%)	**0.032**	35.8% (24.8%–46.7%)	**0.001**
Integrated discrimination improvement				
suPAR in addition to NT-proBNP	0.022 (0.014–0.028)	**0.008**	0.060 (0.047–0.073)	**<0.001**
suPAR in addition to multivariate model	0.011 (0.006–0.017)	**0.05**	0.022 (0.013–0.030)	**0.01**

Harrell's C-statistic was calculated to compare the discriminatory power to predict mortality of soluble urokinase-type plasminogen activator receptor in addition to N-terminal pro B-type natriuretic peptide and a multivariate model comprising all variables independently associated with mortality in multivariate Cox regression (age, male sex, New York Heart association functional classification and N-terminal pro B-type natriuretic peptide). An improvement in individual risk stratification was assessed using the category free net reclassification index. The p values in **bold** indicate a value <0.05.

AUC = area under the curve; other abbreviations as in Tables 1 and 2.

study, CRP showed a significant association with mortality in univariate Cox regression, which became nonsignificant after adjustment for suPAR, whereas the association of suPAR with mortality remained (Online Tables 2 and 3). This may be explained by the high stability of suPAR in plasma resulting in a better reflection of the state of chronic immune activation than CRP or may be the result of further mechanisms affecting plasma concentrations of suPAR (22,26). Future studies are necessary to reveal whether suPAR directly affects myocardial function or acts as surrogate marker of inflammatory processes.

Several prognostic biomarkers associated with myocardial stretch, inflammation, remodeling, and/or fibrosis, have been investigated in recent years in order to refine the risk stratification of patients with CHF. sST2 is one of the few markers which integrates these pathophysiological processes and provides additional prognostic information beyond NT-proBNP levels (14–16). In the present study, sST2 was also independently associated with all-cause mortality and cardiovascular mortality in clinical multivariate models and circulating levels of sST2, and suPAR showed a significant positive correlation. Because each new marker must add prognostic information on top of currently used parameters, we compared the predictive value of suPAR with that of sST2 (17). SuPAR had a higher crude HR per 1 SD, which is a convenient approach with which to compare the predictive value of continuous variables. In the fully adjusted multivariate model, including demographics, clinical variables, and biomarkers, only suPAR independently predicted all-cause mortality, whereas sST2 lost its association with mortality. These data suggest that suPAR may be superior for risk stratification in CHF compared to that in sST2.

When analyzing the associations of suPAR with clinical variables, we observed a strong correlation (cc = 0.6) with impaired renal function, which is in line with several studies linking suPAR to chronic kidney disease (7,27). Recent reports indicate a direct involvement of elevated suPAR levels in glomerular damage. Hayek et al. (6) showed a more pronounced decline of eGFR-associated with higher levels of suPAR, particularly in patients with normal renal function at baseline, which may be indicative of a causal role of suPAR in renal injury. Corroborating evidence derives from experimental studies showing that suPAR interacts with integrins on podocytes promoting an increased glomerular damage (28,29). Due to the strong correlation with eGFR, it is tempting to speculate that suPAR merely reflects the extent of renal impairment in CHF, a well-established prognosticator in this group of patients (8). Correspondingly, in our cohort, eGFR and creatinine levels were associated with mortality in univariate Cox regression but were clearly outperformed by suPAR after adjustment (Online Tables 2 and 3). The question remains whether suPAR represents a more sensitive marker of existing and progressing renal impairment in CHF or, more likely, may reflect multiple pathophysiologic pathways as aforementioned.

STUDY LIMITATIONS. The derivation and validation cohorts were recruited according to different inclusion criteria. The derivation cohort consisted of patients from an outpatient clinic for CHF in stable condition, whereas the validation cohort consisted of patients after decompensated CHF. As a result, the validation cohort consisted of older patients in more advanced stages of the disease and with a higher load of comorbidities, which may limit the comparability of the results. However, as we observed similar results regarding the predictive value of suPAR in both cohorts, this might also have enhanced the generalizability of the study, as results can be applied to a broader spectrum of patients with CHF. A further limitation of the study may be the rather low number of patients. However, a high number of hard endpoints and long follow-up periods and validation in the second cohort with largely corresponding results should provide sufficient power and overcome statistical concerns. Although the association of suPAR with hard endpoints (all-cause mortality) was striking, the study lacks data for rehospitalization due to heart failure and non-fatal cardiovascular events. Future studies are necessary to assess the association of suPAR with these endpoints.

CONCLUSIONS

Our study demonstrated that suPAR may be a promising candidate for improved risk prediction in patients with CHF. Although suPAR was associated with a number of risk factors including impaired renal function, elevated CRP and NT-proBNP levels, age, higher NYHA functional class, and common CHF-related comorbidities such as type 2 diabetes mellitus, hypertension, and AF, it may represent an integrative biomarker for a variety of organ and tissue dysfunctions with excellent prognostic value. SuPAR revealed prognostic superiority over sST2 and remained predictive even after comprehensive adjustment for potential confounders and provided

additional prognostic insights compared with that available with conventional cardiovascular risk factors. SuPAR thereby fulfills all quality criteria recommended by Ahmad et al. (17) for the evaluation of a novel prognostic biomarker in CHF, including accurate methodological and statistical procedures, gain in prognostic information beyond clinically available information, validation in a second cohort, and comparison with further biomarkers. However, future studies using an integrative approach to investigate specific clinical aims like response to therapy and comparison with a multiplicity of biomarkers are warranted to further assess the clinical applicability for suPAR and to establish an individualized biomarker-based treatment in patients with CHF. It is further tempting to speculate that future therapies targeting complementary pathophysiologic systems in CHF like inflammation will need specific biomarkers representing the respective pathways.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: As chronic heart failure (CHF) is associated with high rates of adverse events and poor prognosis, risk prediction is crucial in this vulnerable group of patients. In the present study, we observed that determination of the inflammatory marker soluble urokinase-type plasminogen activator receptor (suPAR) added incremental prognostic value to established risk prediction strategies in CHF. Implementation of suPAR into clinical practice may therefore help to improve risk assessment in CHF and to identify patients at very high risk, who might profit from intensified treatment procedures.

TRANSLATIONAL OUTLOOK: Immune activation and inflammation are substantially involved in the pathophysiology of CHF. SuPAR may represent a promising target for future therapeutic approaches in these patients. Determination of suPAR may furthermore be useful to monitor future anti-inflammatory therapies due to its stable character and strong association with prognosis in CHF.

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APPENDIX For supplemental tables and a figure, please see the online version of this article.