Soluble Urokinase Plasminogen Activator Receptor (suPAR) and All-Cause and Cardiovascular Mortality in Diverse Hemodialysis Patients

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Claudia Torino[1], Patrizia Pizzini[1], Sebastiano Cutrupi[1], Maurizio Postorino[2], Giovanni Tripepi[1], Francesca Mallamaci[1,2], Jochen Reiser[3] and Carmine Zoccali[1]; on behalf of the PROGREDIRE Working Group

[1]CNR-IFC, Clinical Epidemiology and Physiopathology of Renal Diseases and Hypertension Unit, Reggio Calabria, Italy; [2]Nephrology and Renal Transplantation Unit, Reggio Calabria, Italy; and [3]Department of Medicine, Rush University Medical Center, Chicago, Illinois, USA

Introduction: The soluble receptor of urokinase plasminogen activator (suPAR) is an innate immunity/inflammation biomarker predicting cardiovascular (CV) and non-CV events in various conditions, including type 2 diabetic patients on dialysis. However, the relationship between suPAR and clinical outcomes in the hemodialysis population at large has not been tested.

Methods: We measured plasma suPAR levels (R&D enzyme-linked immunosorbent assay [ELISA]) in 1038 hemodialysis patients with a follow-up of 2.9 years (interquartile range = 1.7–4.2) who were enrolled in the PROGREDIRE study, a cohort study involving 35 dialysis units in 2 regions in Southern Italy.

Results: suPAR was strongly ($P < 0.001$) and independently related to female gender ($\beta = -0.160$), age ($\beta = 0.216$), dialysis vintage ($\beta = 0.264$), CV comorbidities ($\beta = 0.105$), alkaline phosphatase ($\beta = 0.136$), albumin ($\beta = -0.147$), and body mass index (BMI; $\beta = 0.174$) (all $P < 0.006$). In fully adjusted analyses, suPAR tertiles predicted the risk of all-cause mortality (third tertile vs. first tertile hazard ratio (HR) = 1.91, 95% confidence interval (CI) = 1.47 – 2.48, $P < 0.001$), CV mortality (HR = 1.47, 95% CI = 1.03–2.09, $P = 0.03$), and non-CV mortality (HR = 1.94, 95% CI = 1.28–2.93, $P = 0.002$); these relationships were not modified by diabetes or other risk factors. suPAR added only modest prognostic risk discrimination and reclassification power for these outcomes to parsimonious models based on simple clinical variables.

Conclusion: In conclusion, suPAR robustly predicted all-cause and both CV and non-CV mortality in a large unselected hemodialysis population. Intervention studies are needed to definitively test the hypothesis that suPAR is causally implicated in clinical outcomes in this population.

I n 2010, more than 2.5 million people with end-stage kidney disease (ESKD) were on chronic dialysis treatment worldwide, and the number of ESKD on dialysis is projected to more than double by 2030.[1] The prognosis of these patients is notoriously dismal, with a death rate of about 20% per year among patients on stable hemodialysis treatment, both in the United States[2] and in Europe.[3] Most clinical trials aimed at testing interventions targeting the excess death risk of ESKD have been disappointing,[4] and reducing the high mortality rate in the dialysis population—an inherently multifactorial issue[5]—remains a largely unmet clinical need.

Among the risk factors implicated in the high risk of death from ESKD, altered innate immunity/inflammation has emerged as a major risk factor for the systemic complications of this condition.[6,7] In general, studies looking at the link between innate immunity/inflammation and mortality and/or cardiovascular (CV) disease have been based mainly on C-reactive protein (CRP),[8] whereas only a minority of studies applied interleukin-6 (IL-6) and other innate immunity biomarkers. Soluble urokinase plasminogen activator receptor (suPAR) is 1 of the strongest biomarkers of

Correspondence: Carmine Zoccali, CNR-IFC c/o EUROLINE S.r.l. - Via Vallone Petrara 57-59, 89124 Reggio Calabria, Italy. E-mail: carmine.zoccali@tin.it

innate immunity activation. and several studies have documented that high suPAR levels consistently associates with mortality in septic patients in intensive care units[9,10] as well as with death and CV events in various populations,[11–13] including patients with mild to moderate CKD.[14] This biomarker directly orchestrates fundamental processes in the pathogenesis of atherosclerosis, including monocyte adhesion to the endothelium, migration, and proliferation,[9] and it is more strongly associated with vascular damage and adverse clinical outcomes than classical innate immunity/inflammation markers such as CRP. Indeed, suPAR is superior to CRP for predicting CV disease events in high-risk patients.[15] suPAR appears to be of particular relevance in CKD because it is not only a likely causal risk factor for renal damage[16] but also a powerful prognostic factor predicting faster renal function loss in patients with suspected CV disease and normal glomerular filtration rate (GFR).[17] suPAR levels are, on average, markedly raised in ESKD patients maintained on chronic hemodialysis treatment.[18] For the foregoing, a thorough assessment of the relationship between suPAR and all-cause and CV mortality appears to be of obvious relevance in ESKD. The sole study in ESKD patients performed so far was based on the 4D Study, a landmark clinical trial testing the effect of atorvastatin in type 2 diabetic patients maintained on chronic dialysis.[18] Observational analyses in the framework of clinical trials as in the 4D Study are an unquestionable source of information for generating new hypotheses about the etiology and prognosis; however, findings in these studies are restricted to the specific population of these trials, for example, type 2 diabetic patients in the 4D Study.

Until now, there has been no cohort study in the dialysis population investigating the relationship between suPAR and mortality in unselected patients on chronic dialysis. With this background in mind, we investigated the relationship between suPAR and all-cause and CV mortality in a large cohort of dialysis patients, including the vast majority of the dialysis population in a region of Italy with approximately 2 million inhabitants.

METHODS

The study protocol was approved by the ethical committee of our institution. All participants gave their informed consent before enrollment.

Study Population

The study population is part of a cohort of 1189 dialysis patients enrolled from February 2009 to October 2010 in the Prospective Registry of The Working Group of Epidemiology of Dialysis Region Calabria (PROGREDIRE), a cohort study involving 35 dialysis units in 2 regions in southern Italy (Calabria and Sicily) and covering a geographical area with approximately 2 million residents. We included in this analysis 1038 hemodialysis (HD) patients in whom suPAR measurements could be performed. Patients had been on regular HD for a median time of 3.8 years (interquartile range [IQR] = 1.8−7.4 years) and were being treated with standard bicarbonate dialysis with noncellulosic membrane filters of various type. Arteriovenous fistula was the vascular access most commonly used (in 86.3% of patients). Patients were on dialysis 3 ± 0.4 times a week, with a mean dialysis time of 233 ± 18 minutes and a blood flux of 288 ± 33 ml. A total of 559 patients were treated with various antihypertensive drugs (240 on mono-therapy with angiotensin-converting enzyme inhibitors, calcium channel blockers, α- and β-blockers, vasodilators, diuretics, or other drugs, with 188 on double therapy, 73 on triple therapy, and 58 patients on quadruple or quintuple therapy with various combinations of these drugs). The main demographic, somatometric, clinical, and biochemical characteristics of the study population are detailed in Table 1. A total of 88 patients were censored at the time of renal transplantation and 50 patients because they were lost to follow-up.

Laboratory Measurements

Blood sampling was performed at baseline after an overnight fast. Blood was drawn with ethylenediamine tetraacetic acid as an anticoagulant during a mid-week day (brief dialysis interval). suPAR was assayed by enzyme-linked immunosorbent assay (R&D Systems, Inc., Minneapolis, MN) from banked (at −80°), never-defrosted specimens. At baseline, we separated collected serum in several aliquots to prevent defreezing/refreezing cycles and sample deterioration. The coefficient of variation of this assay was 4.6% (intra-assay) and 5.5% (interassay). The upper limit of the normal range of plasma suPAR by this method in the healthy population is 3829 pg/ml (Quantikine ELISA human uPAR immunoassay; R&D Systems, Minneaplis, MN) Cholesterol, albumin, calcium, phosphate, alkaline phosphatase, parathyroid hormone (intact molecule), CRP, fibrinogen, and hemoglobin measurements were made using standard methods in the routine clinical laboratory. Residual renal function was estimated according to Stel *et al.*, that is, by calculating the residual estimated glomerular filtration rate (eGFR).[19]

Study Endpoints

Mortality and CV mortality, fatal CV events, and nonfatal CV events were the main study endpoints. Cardiovascular events were centrally adjudicated and

Table 1. Main demographic, somatometric, and clinical characteristics in the whole study population and in patients divided according to suPAR tertiles

Characteristics	Whole group (N = 1038)	suPAR tertiles			Among-groups comparison P	r (P) In suPAR versus
		First tertile (n = 351)	Second tertile (n = 336)	Third tertile (n = 351)		
Age, yr	65 ± 14	61 ± 15	66 ± 12	68 ± 12	<0.001	r = 0.222, P < 0.001
Body mass index, kg/m²	25 ± 5	24 ± 4	25 ± 4	26 ± 5	<0.001	r = 0.121, P < 0.001
Waist circumference, cm	98 ± 14	95 ± 13	98 ± 14	100 ± 14	<0.001	r = 0.129, P < 0.001
Male sex, n (%)	662 (64)	258 (74)	217 (65)	187 (53)	<0.001	r = −0.172, P < 0.001
Smoker, n (%)	141 (14)	58 (17)	46 (14)	37 (11)	0.08	r = −0.038, P=0.226
Diabetic, n (%)	281 (27)	71 (22)	98 (31)	112 (34)	0.001	r = 0.088, P = 0.006
Dialysis vintage, mo	46 (21–88)	35 (16–67)	45 (21–89)	62 (28–114)	<0.001	r = 0.220, P < 0.001
With central catheter or arterial graft, n (%)	129 (14)	33 (10)	37 (12)	59 (20)	0.004	r = 0.082, P = 0.01
Kt/V	1.29 ± 0.42	1.29 ± 0.39	1.29 ± 0.49	1.28 ± 0.39	0.99	r = 0.026, P = 0.45
With cardiovascular comorbidities, n (%)	525 (51)	142 (41)	179 (53)	204 (59)	<0.001	r = 0.156, P < 0.001
Coronary heart disease,[a] n (%)	179 (17)	44 (13)	62 (19)	73 (21)	0.012	r = 0.089, P = 0.004
Heart failure	113 (15)	20 (8)	46 (18)	47 (18)	0.001	r = 0.119, P < 0.001
Cerebrovascular disease,[b] n (%)	117 (11)	38 (11)	37 (11)	42 (12)	0.88	r = 0.046, P = 0.14
Peripheral vascular disease, n (%)	197 (19)	44 (13)	62 (19)	91 (26)	<0.001	r = 0.117, P < 0.001
On antihypertensive treatment, n (%)	559 (54)	200 (57)	186 (56)	173 (50)	0.014	r = −0.106, P = 0.001
Systolic blood pressure, mm Hg	135 ± 22	136 ± 20	136 ± 23	133 ± 23	0.18	r = −0.076, P = 0.02
Diastolic blood pressure, mm Hg	74 ± 12	76 ± 11	73 ± 12	72 ± 12	<0.001	r = −0.160, P < 0.001
Cholesterol, mg/dl	155 ± 39	153 ± 37	156 ± 40	155 ± 42	0.55	r = 0.041, P = 0.21
Hemoglobin, g/dl	11.3 ± 1.5	11.3 ± 1.4	11.3 ± 1.5	11.2 ± 1.5	0.83	r = −0.014, P = 0.67
Leukocyte count, 10³/ml	6.7 ± 2.0	6.5 ± 1.8	6.8 ± 2.0	6.9 ± 2.2	0.04	r = 0.066, P = 0.04
Albumin, g/dl	3.9 ± 0.5	4.0 ± 0.5	3.9 ± 0.5	3.8 ± 0.6	0.002	r = −0.138, P < 0.001
C-reactive protein, mg/l	5.0 (3.0–12.0)	4.0 (3.0–9.0)	5.0 (3.0–13.8)	6.0 (3.0–15.0)	0.005	r = 0.073, P = 0.003
Fibrinogen, mg/dl	385 ± 110	363 ± 97	401 ± 112	392 ± 116	<0.001	r = 0.115, P = 0.001
Calcium, mg/dl	9.1 ± 0.9	9.2 ± 0.9	9.2 ± 0.9	9.1 ± 1.0	0.17	r = −0.056, P = 0.09
Phosphate, mg/dl	5.0 ± 1.6	5.2 ± 1.6	4.9 ± 1.6	5.0 ± 1.8	0.13	r = -0.043, P = 0.19
Parathyroid hormone, pg/ml	239 (116–460)	239 (122–494)	252 (119–463)	226 (109–440)	0.38	r = −0.021, P = 0.53
Alkaline phosphatase, UI/l	85 (65–116)	75 (59–97)	85 (64–123)	98 (77–145)	<0.001	r = 0.161, P < 0.001

Data are expressed as mean ± SD, as median and interquartile range, or as percent frequency, as appropriate. Comparisons among groups were made by one-way analysis, the Kruskal–Wallis test, or the χ² test, as appropriate. Linear correlation for continuous variables and point biserial correlation were applied to correlate continuous and binary variables, respectively. Bold values indicate significant correlations.
[a]Past myocardial infarction or angina, coronary angioplasty, or surgery.
[b]Stroke or transient ischemic attack.

classified as follows: stroke (ischemic or hemorrhagic), documented by computed tomography, magnetic resonance imaging, and/or clinical and neurological evaluation; transient ischemic attacks; myocardial infarction confirmed by serial changes of electrocardiographic and cardiac biomarkers; electrocardiographically documented angina episodes; electrocardiographically documented arrhythmia; and unexpected, sudden death highly suspected as of cardiac origin. *De novo* chronic heart failure was defined as chronic heart failure in a patient without this condition at baseline. To be classified as having chronic heart failure, patients had to show mild or more severe dyspnea during ordinary activities (New York Heart Association class II or higher) plus evidence of anatomical/functional left ventricular disease on echocardiography. Each cause of death was assessed by 3 independent physicians. In doubtful cases, diagnosis was attributed by consensus. During the review process, the involved physicians used all available medical information, including hospitalization forms and medical records. In case of death occurring at home, family members and/or general practitioners were interviewed to better understand the circumstances that led to death.

Statistical Analysis

Data were expressed as mean ± SD (normally distributed data), median and IQR (non–normally distributed data), or as percent frequency (categorical data). Comparisons among groups were made by 1-way analysis of variance, the Kruskal–Wallis test, or the χ² test, as appropriate. Pearson correlation analyses were performed to investigate the correlates of suPAR. Independent correlates of suPAR were investigated by including, in a linear regression model, all variables associated with this biomarker in correlation analyses. Because of the nonnormal distribution of suPAR, this variable was log transformed before analysis. The final functional form of suPAR for survival analyses was investigated by using Martingale residuals.[20] This analysis showed 2 steps at 5625 and 6999 pg/ml, corresponding approximately to the upper limits of the

first and second tertiles. We therefore adopted suPAR categorization by tertiles for subsequent analyses. Survival analyses were performed by using both univariate and multivariate Cox regression analyses, including suPAR as a categorical (tertiles) variable, traditional (age, gender, current smoking, diabetes, cholesterol, arterial pressure, antihypertensive treatment, and CV comorbidities), inflammation and nutritional status (CRP, albumin, fibrinogen, body mass index [BMI]), and ESKD-related risk factors (dialysis vintage, Kt/V, fistula/catheters or grafts, hemoglobin, parathyroid hormone, alkaline phosphatase). The number of missing values was less than 10% for each variable. In multiple models, we adopted the most conservative approach to replace missing data, that is, we replaced missing data by the mean or median values (according to the data distribution). The date of renal transplantation or last observation for patients lost to follow-up was carefully recorded. As these events were not the endpoint of the study, these patients were considered as censored in both models. Survival curves were made to compare the risk of the considered outcome. The association between suPAR and CV or non-CV mortality was assessed by using competitive risk regression models.[21,22] Nonlinear associations were assessed by estimating hazard ratios of mortality and CV mortality according to suPAR with its linear spline terms[23] with knots at 5625 and 6999 pg/ml. The potential effect modification by diabetes, somatometric measurements (BMI), dialysis vintage, blood pressure and antihypertensive drug use, biomarkers of protein-energy wasting and inflammation (albumin, CRP, fibrinogen) and Kt/V on the relationship between suPAR and death/CV death was investigated by standard analyses by creating appropriate multiplicative terms in Cox regression analyses or in competitive risk regression models, as appropriate. The prognostic power of suPAR was assessed by using the Harrell C Index, the Net Reclassification Index, and the Integrated Discrimination Index. In each test, we compared a parsimonious model based on backwardly selected variables (backward selection concluded after 13 steps for all-cause mortality, 15 steps for CV mortality) with a full model, including the same variables as in the base model and suPAR as categorical variable. For the Net Reclassification Index, the cut-offs for defining risk categories (all-cause death: <34%, 34%−51%, >51%; CV death: <17%, 17%−30%, >30%) were chosen according to the distribution of the predicted probability of death/CV death. Statistical analysis was performed by using standard statistical packages (SPSS for Windows, Version 24; SPSS Inc., Chicago, Illinois, USA; STATA for Windows, Version 13; Stata Corp., College Station, Texas, USA).

RESULTS

The main baseline characteristics of the study population are reported in Table 1. The median value of suPAR was 6250 pg/ml (IQR = 5319−7633). In all, 64% of patients were male, and the mean age was 65 years. A total of 281 patients were diabetic (27%). The cause of ESKD was hypertension-related nephropathy in 321 patients (31%), glomerulonephritis in 129 (12%), pyelonephritis or interstitial nephritis in 80 (8%), autosomal dominant polycystic kidney disease or other genetic diseases in 120 (12%), and unknown in 192 (19%). suPAR levels were higher in female patients (median = 6605 pg/ml, IQR = 5666−8303 pg/ml) than in male patients (median = 6043 pg/ml, IQR = 5120−7144 pg/ml) and exceeded the upper limit of the normal range (3829 pg/ml) in the vast majority of patients (1007 of 1038, 97%). In Table 1, patients are presented by suPAR tertiles. Patients with higher levels of suPAR (third tertile) were older, with a higher BMI and waist circumference, were more frequently diabetic, and had a higher burden of CV comorbidities and a longer dialysis vintage as compared to patients in the other tertiles. They were also more frequently dialyzed by a central catheter or an arterial graft (Table 1). Furthermore, the same patients exhibited deranged levels of protein-energy wasting and inflammation biomarkers (lower serum albumin and higher CRP, leucocyte count, and fibrinogen), and had higher alkaline phosphatase and lower systolic and diastolic blood pressure. The use of antihypertensive agents was less frequent in the third than in the other two suPAR tertiles. These associations were fully confirmed in correlation analyses (Table 1, last column).

Independent Correlates of suPAR

On multiple regression analysis, including all significant correlates of suPAR (Table 1), only gender (β = −0.160), age (β = 0.216), dialysis vintage (β = 0.264), CV comorbidities (β = 0.105), alkaline phosphatase (β = 0.136), albumin (β = −0.147), and BMI (β = 0.174, all $P \leq 0.006$) maintained an independent association with suPAR.

Survival Analysis

During a median follow-up of 2.9 years (IQR = 1.7≤4.2 years), 436 patients died, 247 of them of CV causes. The causes of death are shown in Supplementary Table S1. A categorical analysis by suPAR tertiles (see Methods) showed a dose−response relationship between suPAR tertiles and risk of death (Figure 1), and this relationship held true after adjustment for traditional, inflammation, nutritional status, and ESKD-related risk factors (third tertile vs. first tertile HR = 1.84, 95% CI = 1.42–2.39, $P < 0.001$). Notably, the HR remained
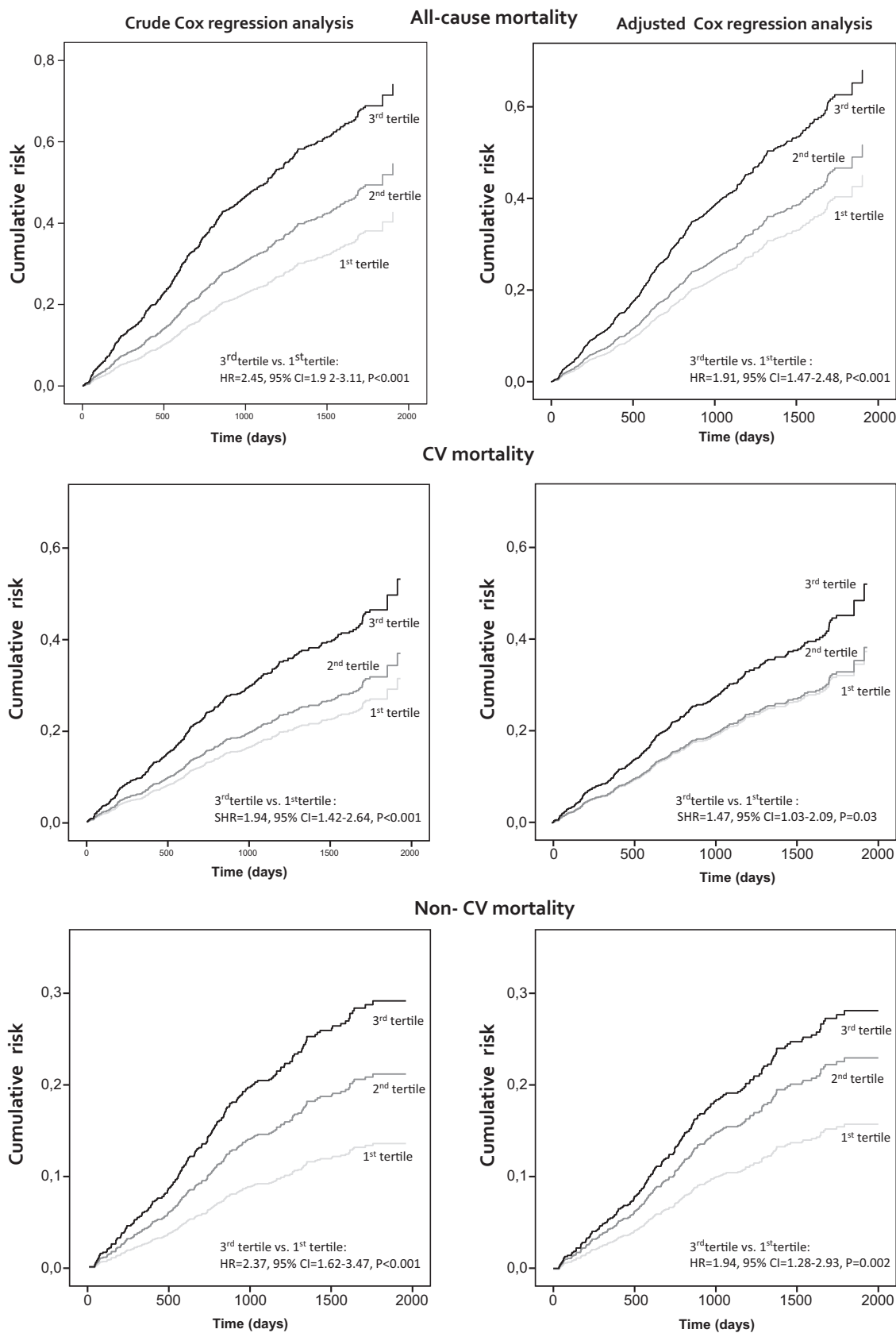
Figure 1. Crude and adjusted Cox regression analysis showing the effect of soluble urokinase plasminogen activator receptor (suPAR) on all-cause, cardiovascular, and noncardiovascular mortality, respectively. Variables included are suPAR and all variables listed in Table 1. Survival curves were made to compare the risk of the considered outcome. The association between suPAR and cardiovascular (CV) or non-CV mortality was assessed by using competitive risk regression models (see Materials and Methods for further details). CI, confidence interval; HR, hazard ratio; SHR, subdistribution hazard ratio.

the same after exclusion of albumin (1.85, 95% CI = 1.43−2.40, $P < 0.001$) or CRP (1.83, 95% CI = 1.41−2.37, $P < 0.001$) or both of these factors (1.84, 95% CI = 1.42−2.39, $P < 0.001$) from the Cox model (Supplementary Table S2), suggesting that the relationship between suPAR and all-cause death is independent of these biomarkers of inflammation.

The effect of suPAR was also tested for CV mortality and non-CV mortality separately, by using competing risk models. As shown in Figure 1, the risk of CV mortality was highest in the third suPAR tertile, and this relationship was confirmed in fully adjusted analyses (third tertile vs. first tertile subdistribution: HR = 1.48, 95% CI = 1.04–2.10, $P = 0.03$), and an even stronger association was observed for non-CV mortality (third tertile vs. first tertile subdistribution hazard ratio = 1.89, 95% CI = 1.24–2.87, $P = 0.003$). Comparison of HR of suPAR (third tertile vs. first tertile) for

non-cardiovascular and cardiovascular mortality obtained by standard Cox regression analysis and competing risk regression analysis are shown in Supplementary Table S3. Similarly, analysis of the association of suPAR with all-cause and CV mortality by nonlinear models (see Methods) again confirmed robust links between suPAR and these outcomes (Figure 2).

To investigate whether the link between suPAR and clinical outcomes differed in diabetic and nondiabetic patients, we performed an effect modification analysis by this disease. As shown in Figure 3, the hazard rates for all-cause (nondiabetic: 1.48; 95% CI = 1.25−1.75; diabetic: 1.31, 95% CI = 1.06−1.62, $P = 0.36$), CV (nondiabetic 1.23, 95% CI = 0.96−1.57); diabetic: 1.25, 95% CI = 0.96−1.62, $P = 0.92$), and non-CV (nondiabetic: 1.54, 95% CI = 1.22−1.94; diabetic: 1.15, 95% CI = 0.79−1.68, $P = 0.19$) death were almost identical in diabetic and nondiabetic patients with no effect
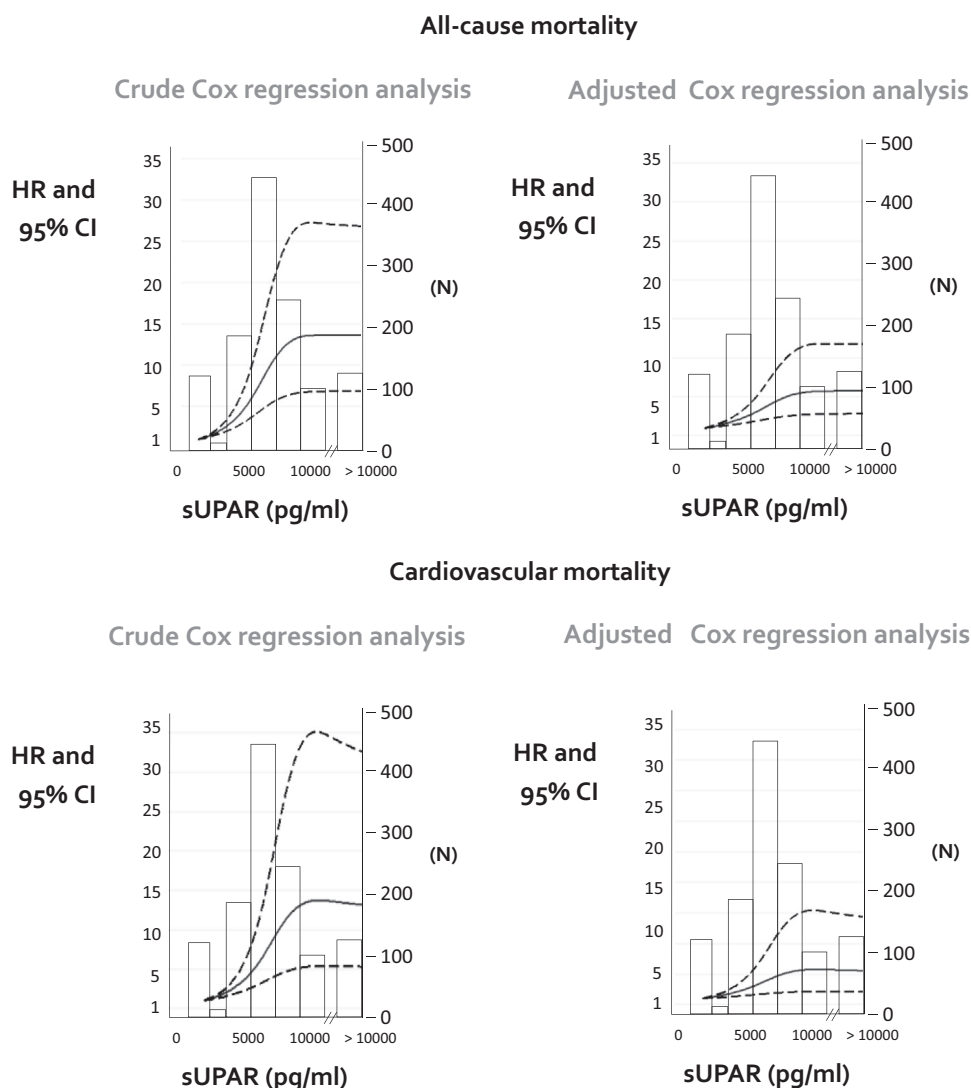

Figure 2. Analysis of the association of soluble urokinase plasminogen activator receptor (suPAR) with all-cause and cardiovascular (CV) mortality by nonlinear models. Models were adjusted for the full list of variables presented in Table 1. Nonlinear associations were assessed by estimating hazard ratios of mortality and CV mortality according to suPAR with its linear spline terms 36 with knots at 5625 and 6999 pg/ml. (See Methods for further details). n = Number of patients corresponding to specific values of suPAR. CI, confidence interval; HR, hazard ratio.
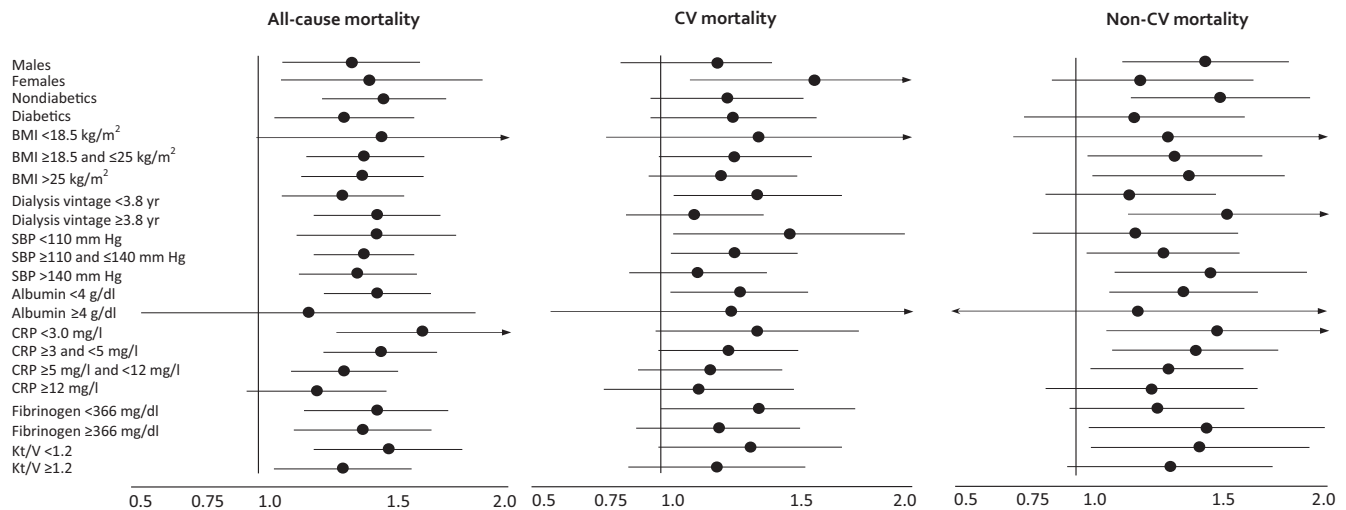
Figure 3. Absence of effect modification attributable to diabetes, body mass index (BMI), dialysis vintage, systolic blood pressure (SBP), albumin, C-reactive protein (CRP), fibrinogen, and Kt/V on the link between soluble urokinase plasminogen activator receptor (suPAR)—considered outcome (all-cause mortality/cardiovascular mortality/noncardiovascular mortality).

modification. Similarly, no effect modification by gender, BMI, dialysis vintage, systolic blood pressure, albumin, CRP, fibrinogen, and Kt/V was registered (Figure 3 and Supplementary Table S4).

Prognostic Power of suPAR

The addition of suPAR to parsimonious models of all-cause and CV mortality in dialysis patients (including age, diabetes, cholesterol, CV comorbidities, dialysis vintage, albumin, vascular access, and alkaline phosphatase for all-cause death; age, diabetes, cholesterol, CV comorbidities, vascular access and alkaline phosphatase for CV death) added only 3% (all-cause death) or 2% (CV death) of explanatory power. The gain in discriminating power, as assessed by the Harrell C Index, was less than 1% for both outcomes. suPAR was also scarcely useful to correctly reclassify the risk categories (all-cause death: Net Reclassification Index = 0.6%, $P = 0.77$; Integrated Discrimination Index = 1%, $P = 0.0007$; CV death: Net Reclassification Index = −0.2%, $P = 0.94$; Integrated Discrimination Index = 0.4%, $P = 0.09$).

DISCUSSION

In a cohort of patients formed by the 80% the dialysis population in a geographical area in Italy with approximately 2 million inhabitants, suPAR was independently associated with female gender, age, dialysis vintage, and background CV comorbidities, as well as with protein energy wasting and inflammation biomarkers. Independently of other factors, suPAR levels were related to the risk of all-cause and both CV and non-CV death in diabetic and nondiabetic patients across various risk factors strata, including diabetes and other risk factors,

suggesting a possible causal implication of this molecule in adverse clinical outcomes in ESKD. On the other hand, suPAR added quite modest predictive power to parsimonious risk models based on standard risk factors in the dialysis population.

The suPAR molecule is one of the most interesting innate immunity signaling molecules and biomarkers in chronic kidney disease.[24] It belongs to the lysine 6—neurotoxin family of signaling proteins known to bind integrins to regulate cell adhesion.[25] Well beyond being causally implicated in focal glomerulosclerosis,[26] suPAR, a 50-kDa protein, associates strongly with the GFR[17] and predicts the incidence of CKD in patients with suspected coronary heart disease[16] and individuals in the general population,[21] as well as the rate of GFR loss in children.[27] We found that in ESKD patients, circulating suPAR, a compound that is not removed by dialysis, exceeded the upper limit of the normal range in the vast majority (97%) of patients in our cohort.

Interest in suPAR rests mainly on the strong relationship between circulating levels of this compound and all-cause and CV mortality and other adverse clinical outcomes in patients with severe multiorgan diseases admitted into intensive care units,[9,10] in the general population,[11] as well as in patients with CV disease,[12,13] cancer,[11] sepsis,[28] and HIV.[29] In the present study, suPAR associated with all-cause, CV, and non-CV mortality in a dose-dependent fashion, and these associations held true both in diabetic and in nondiabetic patients. Thus, the risk implications of high suPAR levels in hemodialysis patients, a population with virtually no renal function, are not confined to diabetic patients[18] but apply to the hemodialysis population at large also

irrespective of the presence of background CV comorbidities and other risk factors. We found that suPAR was independently related to biomarkers of protein-energy wasting and inflammation, a syndromic complex consistently associated with all-cause and CV mortality in ESKD.[30] However, the predictive power of suPAR was completely independent of protein-energy wasting and inflammation as quantified by serum albumin, CRP, and BMI, indicating that inflammatory pathways other than protein-energy wasting and CRP are involved in the high risk of elevated suPAR levels. Urokinase plasminogen activator receptor (uPAR) is a receptor for urokinase and binding partner for integrins produced in various cell species including the endothelium, macrophages, T cells, fibroblasts, and epithelial cells, and circulating suPAR reflects cellular shedding of uPAR.

Recently, the production of suPAR from immature myeloid cells has been shown to be a constant source in focal and segmental glomerulosclerosis.[31] On binding its receptor, uPAR triggers the production plasmin as well as cell adhesion, migration, and proliferation, which are wide-ranging phenomena that participate in atherosclerosis,[32] infectious diseases,[28] and cancer.[11,33]

While suggesting an etiologic role of suPAR in adverse health outcomes in ESKD, our analyses show that this biomarker *per se* adds quite modest predictive power to parsimonious models based on standard risk factors in the ESKD population. Indeed, both discrimination analyses and risk reclassification analyses showed just small gains in the predictive power for all-cause and CV death by adding suPAR to equations based on backwardly selected, simple clinical variables such as age, diabetes, cholesterol, CV comorbidities, vascular access, dialysis vintage, and albumin. This is not surprising, given the plethora of pathophysiological changes that occur in patients with end-stage renal disease. However, our findings that suPAR remains independently predictive of outcomes in this population suggest that the CV manifestations caused by or associated with suPAR do not require functioning kidneys.

Our data remain hypothesis generating and can only suggest, but not prove, causality. Although large, our cohort included white individuals only. Further studies are therefore needed to determin whether the predictive power of suPAR in white hemodialysis patients also applies to other ethnicities, as it does in diseases different from ESKD.[34,35] The possibility that the strong link between suPAR and clinical outcomes in the present study is due to residual confounding by unmeasured variables cannot be excluded. Data in the present study indicate that the link between suPAR and mortality, initially described in type 2 diabetic patients,[18] can be extended to patients with various etiologies of ESKD. In addition, the data imply that high suPAR levels reflect a specific innate-immunity/inflammation pathway in large part independent of the classical inflammatory pathway, including high CRP and hypoalbuminemia, which is eventually conducive to a high risk of death.

In conclusion, suPAR levels robustly predict all-cause and both CV and non-CV mortality in a reasonably large, unselected dialysis population. Interference with uPAR is a potential pathway for countering CV[36] and non-CV[37] events, and therefore our findings may be relevant for designing new studies aimed at curbing the high mortality burden of ESKD.

DISCLOSURE

ACKNOWLEDGMENTS

AUTHOR CONTRIBUTIONS

CZ designed the study and JR contributed with CZ in developing the idea of the same study. CT and MP were responsible for data collection and data analysis and performed statistical analyses with GT. PP and SC performed biochemical measurements and FM gave critical contribution to data analysis and interpretation. CZ has had full access to the data in the study and final responsibility for the decision to submit for publication. CT and CZ wrote the first draft and the final version of the paper that was read and approved by all authors.

SUPPLEMENTARY MATERIAL

Table S1. Main causes of death recorded during the follow-up.
Table S2. HR of all-cause mortality in the fully adjusted model (model 1), in a model without albumin (model 2) or without CRP (model 3) or without albumin and CRP (model 4).

Table S3. Comparison of HR of suPAR (third tertile vs. first tertile) for noncardiovascular and cardiovascular mortality obtained by standard Cox regression analysis and competing risk regression analysis. Both models are adjusted for traditional, inflammation and nutritional status, and ESKD-related risk factors.

Table S4. HR of suPAR for all-cause mortality/cardiovascular mortality/noncardiovascular mortality across gender, diabetes, BMI, dialysis vintage, systolic blood pressure, albumin, CRP, fibrinogen, and Kt/V categories. All *P* values for the effect modification are >0.05.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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