

Soluble urokinase-type plasminogen activator receptor predicts peripheral artery disease severity and outcomes

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Abstract

Soluble urokinase-type plasminogen activator receptor (suPAR) is associated with chronic kidney disease (CKD) severity and peripheral artery disease (PAD). We hypothesize an association of PAD severity and suPAR in patients without advanced CKD and further risk stratification according to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. For study purposes, suPAR was measured in 334 patients with PAD (34% women, age 69 (62–78) years, eGFR 68 ± 20 mL/min/1.72 m²) by commercial ELISA. Patients were followed for 10 years to assess long-term all-cause survival by Cox regression. Higher suPAR levels were associated with lower ankle–brachial index ($R = -0.215$, $p = 0.001$) in patients with PAD without media-sclerosis ($n = 236$). suPAR levels inversely correlated with decreased glomerular filtration rate ($R = -0.476$, $p < 0.001$) and directly correlated with urinary albumin-to-creatinine ratio ($R = 0.207$, $p < 0.001$). Furthermore, higher suPAR levels associated with a higher KDIGO risk score ($p < 0.001$). Baseline suPAR was significantly associated with all-cause mortality (HR 1.40 (95% CI 1.16–1.68), $p < 0.001$) over 10 years. suPAR remained associated with mortality (HR 1.29 (1.03–1.61), $p = 0.026$) after multivariable adjustment for age, sex, cardiovascular risk factors, and eGFR. Future research may define a standard role for suPAR assessment in PAD's work-up and treatment, especially in patients with CKD.

Keywords

atherosclerosis, chronic kidney disease (CKD), mortality, peripheral artery disease (PAD), soluble urokinase-type plasminogen activator receptor (suPAR)

Introduction

Chronic kidney disease (CKD) affects about 11–13% of the world's population and is an emerging public health problem.¹ CKD is currently stratified by a declining glomerular filtration rate and increasing urinary albuminuria.² Patients with CKD display an increased mortality rate mainly due to vascular calcification.^{3,4} Patients suffering from CKD are at increased risk of developing peripheral artery disease (PAD)⁵ and subsequent lower-limb amputation.⁶ Patients with PAD exhibit a reduced all-cause survival and increased risk for cardiovascular events, even in clinically asymptomatic patients.⁷ However, cardiovascular mortality varies among different cohorts of patients with CKD and ideal biomarkers predicting the cardiovascular risk outcomes of patients with CKD are still lacking. Elevated soluble urokinase-type plasminogen activator receptor (suPAR) levels were associated with PAD and PAD-related events.⁸ suPAR is derived from the proteolytic cleavage of urokinase-type plasminogen activator receptor (uPAR) at its glycosylphosphatidylinositol (GPI) anchor site, with bone marrow-derived immature myeloid cells as the main cellular source.^{9,10} Elevated suPAR levels

are associated with various disease entities, including infectious diseases, arthritis, diabetes, cancer, and cardiovascular disease (CVD).^{11–15}

Recent studies focused on the connection between suPAR and different forms of renal diseases, such as focal segmental glomerulosclerosis (FSGS), IgA nephropathy, and diabetic kidney disease.^{16–20} In addition, studies have shown that elevated suPAR levels are an important predictor of incident CKD and decline of renal function in patients suffering from CVD.^{17,21} High levels of suPAR are

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associated with an increased risk of progression to ESRD in Chinese patients with CKD due to glomerulonephritis.²² Furthermore, suPAR has been shown to predict mortality in patients with ESRD on dialysis in European patients.²³

suPAR has been proposed as an inflammatory marker reflecting endothelial dysfunction and subclinical organ damage²⁴ and is expressed in atherosclerotic lesions.²⁵ Recent findings report an association of suPAR and mortality in patients with CAD but failed to link suPAR to the presence or extent of CAD.²⁶ In contrast, higher suPAR levels were reported in the presence of PAD.⁸ We thus hypothesize a possible association of suPAR and lower-extremity PAD severity in patients without severe CKD.

Methods

Study population

suPAR was measured once in 334 patients from the VMC Vienna cohort. Detailed inclusion and exclusion criteria have been published previously.²⁷ In brief, all patients included exhibited stable PAD (asymptomatic or claudication) without planned revascularization. Patients with critical limb ischemia and/or ulceration were excluded. Patients with known cancer or hemodialysis (chronic kidney disease stage 5) were not eligible for this study. The study was approved by the ethics committee of the Medical University of Vienna and complied with the Declaration of Helsinki, including current revisions and Good Clinical Practice guidelines.^{28,29} The procedures followed were in accordance with institutional guidelines, and all subjects gave written informed consent before inclusion into the study.

Definition of cardiovascular comorbidities

Baseline demographic and clinical characteristics were recorded. Hypertension was defined as documentation of systolic blood pressure of ≥ 140 mmHg and/or a diastolic blood pressure of ≥ 90 mmHg in at least two measurements³⁰ or active use of any antihypertensive medication. Diabetes mellitus type 2 was defined as a fasting plasma glucose level over 7.0 mmol/L (126 mg/dL), a glucose level over 11.1 mmol/L (200 mg/dL) after a standardized oral glucose tolerance test,³¹ glycated hemoglobin (HbA1c) over 6.5% (48 mmol/mol), or intake of anti-diabetic medication. Prediabetes was defined by the results of the oral glucose tolerance test as either a fasting plasma glucose of 5.55–6.94 mmol/L (100–125 mg/dL), a 2-hour glucose level of 7.77–11.05 mmol/L (140–199 mg/dL), or an HbA1c of 5.7–6.4% (39–46 mmol/mol). Smoking was defined as current smoking. Former smoking was defined as previous smoking of at least 100 cigarettes. Pack-years were assessed by questionnaire. Body mass index (BMI) was calculated as body weight in kg divided by squared body height in meters (kg/m^2). Fasting blood samples were drawn at baseline for glucose HbA1c, cholesterol, liver, and renal function parameter monitoring. Spot urinary albumin-to-creatinine ratio (UACR) in > 30 mg/g were classified as micro-albuminuria and > 300 mg/g as

macro-albuminuria. UACR measurements were missing in 16 patients. The estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology (CKD-EPI) equation.³²

Definition of PAD

Presence of PAD was detected by noninvasive Doppler sonographic measurements (VL5000; ELCAT, Wolfraatshausen, Germany) by trained technicians. Systolic blood pressure was measured in both arms (brachial arteries) and both ankles (dorsal pedal arteries and posterior tibial arteries). Ankle-brachial index (ABI) was calculated according to the TASC criteria³³ by dividing the higher ankle pressure by the most elevated brachial pressure. In the case of incompressible ankle arteries ($\text{ABI} > 1.4$), patients were classified as media-sclerosis. PAD was classified after the Fontaine classification system by the self-reported pain-free walking distance (52.7% Fontaine stage I, 47% Fontaine stage II, one patient not classified due to orthopedic immobility).

Definition of KDIGO risk categories

Patients were classified for CKD combining eGFR and UACR according to the 2012 KDIGO (Kidney Disease: Improving Global Outcomes) guidelines for the evaluation and management of CKD ranging from CKD G1 A1 ($\text{eGFR} \geq 90$ mL/min and $\text{UACR} < 30$ mg/g) to CKD G5 A3 ($\text{eGFR} < 15$ mL/min and $\text{UACR} > 300$ mg/g).³⁴ Furthermore, patients were divided into four categories for the CKD progression risk, ranging from low risk ($\text{eGFR} \geq 60$ mL/min and $\text{UACR} < 30$ mg/g) to moderate risk ($\text{eGFR} 45\text{--}59$ mL/min, or $\text{eGFR} \geq 60$ mL/min and $\text{UACR} 30\text{--}300$ mg/g) to high risk ($\text{eGFR} 30\text{--}44$ mL/min and $\text{UACR} < 30$ mg/g, or $\text{eGFR} 45\text{--}59$ mL/min and $\text{UACR} 30\text{--}300$ mg/g, or $\text{eGFR} \geq 60$ mL/min and $\text{UACR} > 300$ mg/g) to very high risk ($\text{eGFR} < 30$ mL/min, or $\text{eGFR} < 45$ mL/min and $\text{UACR} 30\text{--}300$ mg/g, or $\text{eGFR} < 60$ mL/min and $\text{UACR} > 300$ mg/g), as stated in the current KDIGO guidelines.³⁴

Sample collection and measurement of suPAR

Fasting blood samples were collected at study entry and stored at -80°C until measurement in 2018. A central freezer surveillance system monitored the storage temperature. Plasma suPAR levels were measured using a sandwich ELISA (suPARnostic kit; ViroGates, Birkerød, Denmark) with a lower detection limit of 0.1 ng/mL, and intra-assay and inter-assay variation of 5.5% and 5.2%, respectively.

Follow-up and outcome

Follow-up of patients was conducted as previously described to identify cardiovascular and PAD-specific events.²⁷ Mortality was assessed by central death registry queries (Statistik Austria). In the case of survival, patients were additionally contacted by phone to ensure data quality. International Classification of Diseases, Tenth Revision

Table 1. Baseline characteristics according to suPAR tertiles.

	Low <i>n</i> = 111	Medium <i>n</i> = 112	High <i>n</i> = 111	<i>p</i> -value
Age, years	66 ± 10	70 ± 10	72 ± 11	< 0.001
Female, <i>n</i> (%)	28 (25.2)	37 (33)	49 (44.1)	0.012
BP systolic, mmHg	143 ± 23	140 ± 20	140 ± 21	0.470
BP diastolic, mmHg	79 ± 10	77 ± 12	76 ± 12	0.065
BMI, kg/m ²	27.9 ± 4.0	27.7 ± 3.6	26.5 ± 4.3	0.015
HbA1c, mmol/mol	41 (38, 49)	42 (39, 51)	43 (38, 48)	0.267
Triglycerides, mg/dL	131 (97, 182)	148 (97, 223)	137 (102, 195)	0.308
HDL-C, mg/dL	51 (45, 65)	51 (42, 58)	51 (43, 63)	0.149
LDL-C, mg/dL	105 (89, 129)	101 (81, 126)	97 (79, 124)	0.353
Statin usage (%)	92 (82.9)	90 (80.4)	84 (74.5)	0.400
CRP, nmol/L	25.7 (12.4, 45.7)	28.6 (15.2, 53.3)	31.4 (15.2, 58.1)	0.080
eGFR, mL/min/1.73 m ²	78.7 ± 15	67.3 ± 18.0	57.5 ± 19.5	< 0.001
UACR, mg/g	9 (5.0, 24.5)	11 (5, 38)	11 (5, 50)	0.086
suPAR, ng/mL	1.81 (1.45, 1.96)	2.47 (2.33, 2.78)	3.60 (3.19, 4.21)	< 0.001
ABI	0.80 ± 0.19	0.76 ± 0.19	0.70 ± 0.18	0.002
Hypertension (%)	102 (91.9)	101 (90.2)	104 (93.7)	0.629
Diabetes (%)	47 (42.3)	51 (45.5)	51 (45.9)	0.823
RAAS blockage (%)	76 (68.4)	78 (70)	86 (77.4)	0.268
Smoking – active (%)	31 (27.9)	38 (33.9)	46 (41.4)	0.105
Coronary artery disease (%)	34 (30.6)	35 (31.3)	37 (33.3)	0.902
Carotid artery disease (%)	41 (36.9)	48 (42.9)	43 (38.7)	0.650

Data are mean ± SD or median (25th, 75th percentile) or *n* (%).

Differences were analyzed by ANOVA and chi-squared test as appropriate. An alpha-level of *p* < 0.05 (two-tailed) was considered statistically significant.

ABI, ankle–brachial index; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (according to the Chronic Kidney Disease Epidemiology equation); HbA1c, glycated hemoglobin A1c, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RAAS, renin-angiotensin aldosterone system; suPAR, soluble urokinase-type plasminogen activator receptor; UACR, urine albumin-to-creatinine ratio.

(ICD-10) codes were retrieved from the central death registry and verified by hospital or autopsy reports as available to quantify cardiovascular mortality.

Statistics

Data are presented as mean ± SD or median (25; 75 percentile). Student's unpaired *t*-test, as well as χ^2 test, were used as appropriate. Differences between multiple groups were analyzed by analysis of variance (ANOVA) or Kruskal–Wallis test as appropriate. An alpha-level of *p* < 0.05 (two-tailed) was considered statistically significant. Variables were log-transformed according to the Kolmogorov–Smirnov test for parametric statistics if needed. Owing to a left-skewed distribution, suPAR levels were log-transformed. Bivariate correlation was estimated using the Pearson correlation coefficient. Survival curves were calculated by the Kaplan–Meier method and compared using the log-rank test. Cox-regression analysis was performed to estimate the effect size and to allow for multivariable adjustment. The effect size for suPAR is given as hazard ratio (HR) per 1 SD and 95% confidence intervals (CI). Multivariable interaction was defined as beta change over 10%.³⁵ A two-sided alpha-level of *p* < 0.05 was considered statistically significant. Kaplan–Meier curves for suPAR tertiles were compared using the log-rank test (*p*-value). All statistical analyses were performed with IBM SPSS

Statistics, Version 24 (IBM Corp., Armonk, NY, USA). The shown Figure 3 was generated by GraphPad Prism 6.0h (GraphPad Software Inc., La Jolla, CA, USA).

Results

This study included 334 elderly patients with PAD (age 69 (62–78) years, 34% women) with suPAR levels of 2.47 (1.96–3.19) ng/mL. Detailed baseline characteristics according to suPAR tertiles are depicted in Table 1. Classical cardiovascular comorbidities such as coronary (*p* = 0.902) or carotid (*p* = 0.650) artery disease were equally distributed among suPAR tertiles at baseline. Furthermore, antihypertensive medication, especially angiotensin-converting enzyme inhibitors (*p* = 0.683) or angiotensin receptor blockage (*p* = 0.521) at the beginning of the study was alike in all suPAR tertiles. During the study period, 127 patients within 9.5 (6.9–10) years died. ICD-10 mortality codes were classified as 78 cardiovascular, 22 oncological, and 27 other causes of death.

suPAR and anthropometric and laboratory parameters

In univariate correlation analyses, suPAR levels were higher in older patients (*R* = 0.279, *p* < 0.001) and in women (*R* = –0.174, *p* = 0.012). suPAR levels were

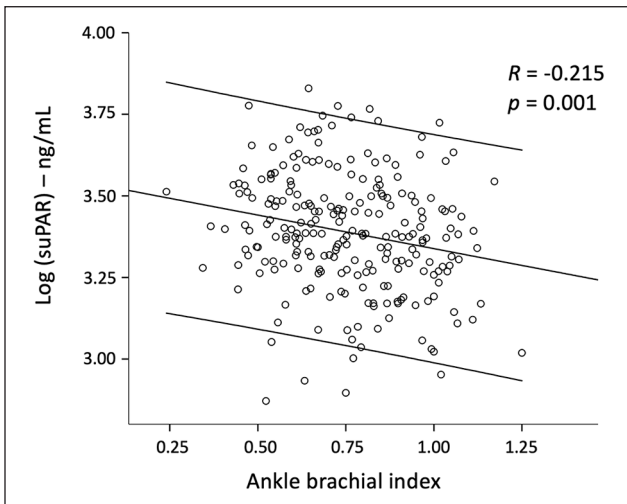


Figure 1. Scatter-plot of the patients' individual suPAR levels and ABI.

R represents Pearson correlation coefficient. An alpha-level of $p < 0.05$ (two-tailed) was considered statistically significant.

ABI, ankle-brachial index; suPAR, soluble urokinase-type plasminogen activator receptor.

inversely associated with bodyweight ($R = -0.193$, $p < 0.001$), also reflected by patients' BMI ($R = -0.133$, $p = 0.015$). However, suPAR was not associated with the waist-to-hip ratio ($R = -0.074$, $p = 0.187$). Patient suffering from type 2 diabetes mellitus (2.59 (2.00, 3.26) ng/mL) showed similar suPAR levels to those without diabetes (2.42 (1.90, 3.15) ng/mL, $p = 0.205$). Baseline laboratory markers of glucose metabolism abnormalities such as fasting glucose ($R = 0.040$, $p = 0.469$) or HbA1c ($R = 0.087$, $p = 0.115$) were not related to suPAR. Fasting total cholesterol ($R = -0.056$, $p = 0.311$), low-density lipoprotein (LDL)-cholesterol ($R = -0.059$, $p = 0.280$), high-density lipoprotein (HDL)-cholesterol ($R = -0.093$, $p = 0.089$), or triglyceride ($R = 0.089$, $p = 0.106$) levels were not associated with suPAR. suPAR was significantly linked to C-reactive protein (CRP) levels ($R = 0.170$, $p = 0.002$).

suPAR and PAD

suPAR levels were associated with lower ABI ($R = -0.215$, $p = 0.001$) in patients with PAD without media-sclerosis ($n = 236$), as shown in Figure 1. The increase in suPAR levels was most distinct in patients with an ABI ≤ 0.5 (0.45 ± 0.14 ng/mL), over ABI ≤ 0.7 (0.44 ± 0.18 ng/mL) to ABI > 0.7 (0.36 ± 0.18 ng/mL) in patients with PAD without media-sclerosis ($p = 0.006$), as shown in online supplemental figure 1. Clinical staging of PAD by the Fontaine classification (I: 52.7%, IIa: 34.1%, IIb: 12.9%) was not associated with suPAR levels ($p = 0.134$).

suPAR and CKD

suPAR levels were higher in patients with worse renal function and were significantly associated with increased serum creatinine levels ($R = 0.373$, $p < 0.001$) and reduced eGFR ($R = -0.476$, $p < 0.001$). Furthermore, suPAR levels were associated with UACR ($R = 0.207$, $p < 0.001$). The

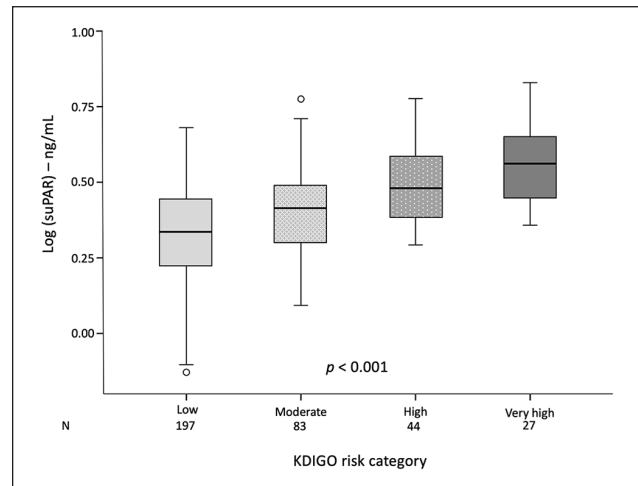


Figure 2. suPAR levels are given according to KDIGO risk for CKD progression category.

Outliers within 1.5 times IQR range below the 1st or above the 3rd quartile are marked by \circ . Differences were analyzed by ANOVA. An alpha-level of $p < 0.05$ (two-tailed) was considered statistically significant. ANOVA, analysis of variance; CKD, chronic kidney disease; KDIGO, Kidney Disease: Improving Global Outcomes; suPAR, soluble urokinase-type plasminogen activator receptor.

combination of eGFR and UACR showed a linear increase of suPAR levels from low to very high risk ($p < 0.001$), as depicted in Figure 2.

suPAR and all-cause mortality

Baseline suPAR levels were significantly associated with all-cause mortality (HR 1.40 (95%CI 1.16–1.68), $p < 0.001$) in patients with PAD over a 10-year observation period. Elevated suPAR levels remained a significant predictor of all-cause mortality after multivariable adjustment for sex, patient age, LDL-cholesterol, systolic blood pressure, type 2 diabetes, and smoking pack-years (HR 1.29 (1.05–1.59), $p = 0.017$). Similar multivariate models exchanging suPAR for eGFR (HR 0.99 (0.98–1.00) or UACR (HR 1.00 (1.00–1.00)) revealed no increased mortality risk in this PAD cohort as depicted in Table 2 (models 1–3). The association of suPAR and all-cause mortality remained significant in a combination model, including risk factors and eGFR (HR 1.29 (1.03–1.61) but not UACR (HR 1.23 (0.98–1.52)) as depicted in Table 2 (models 4 and 5). Survival according to suPAR tertiles was significantly lower in patients with higher suPAR levels, ranging from in the highest (50.5%) over the middle (66.1%) to the lowest (69.4%) suPAR tertile ($p = 0.008$), as depicted in Figure 3. Additionally, there was a numerical increase in cardiovascular mortality in the highest (29.7%), over the middle (22.3%) to the lowest (18%) suPAR tertile ($p = 0.062$).

Discussion

In this study, we found that suPAR levels increased with reduced ABI in patients with PAD without critical limb ischemia. Furthermore, suPAR levels were associated with higher grades of CKD and, therefore, increased risk for CKD progression according to the current KDIGO

Table 2. Multivariable model for all-cause mortality – estimates for covariates.

	Covariate	Hazard ratio (95% CI)	p-value
Model 1: suPAR	suPAR	1.29 (1.05–1.59)	0.017
	Age	1.05 (1.03–1.08)	< 0.001
	Sex	1.37 (0.92–2.05)	0.119
	Pack-years	1.06 (1.00–1.01)	0.015
	LDL-C	1.00 (0.99–1.01)	0.887
	BPs	1.01 (1.00–1.02)	0.119
	T2DM	0.74 (0.51–1.06)	0.100
Model 2: eGFR	eGFR	0.99 (0.98–1.00)	0.218
	Age	1.05 (1.03–1.08)	< 0.001
	Sex	1.39 (0.93–2.04)	0.111
	Pack-years	1.00 (1.00–1.01)	0.062
	LDL-C	1.00 (1.00–1.01)	0.785
	BPs	1.01 (1.00–1.02)	0.125
	T2DM	0.72 (0.51–1.09)	0.062
Model 3: UACR	UACR	1.00 (1.00–1.00)	0.002
	Age	1.05 (1.03–1.08)	< 0.001
	Sex	1.35 (0.90–2.00)	0.144
	Pack-years	1.00 (1.00–1.01)	0.334
	LDL-C	1.00 (1.00–1.01)	0.924
	BPs	1.01 (1.00–1.01)	0.268
	T2DM	0.73 (0.51–1.05)	0.087
Model 4: suPAR + eGFR	suPAR	1.29 (1.03–1.61)	0.026
	Age	1.05 (1.03–1.08)	< 0.001
	Sex	1.37 (0.92–2.05)	0.119
	Pack-years	1.01 (1.00–1.01)	0.015
	LDL-C	1.00 (0.99–1.01)	0.887
	BPs	1.01 (1.00–1.02)	0.119
	T2DM	0.74 (0.51–1.06)	0.101
Model 5: suPAR + UACR	eGFR	1.00 (0.99–1.01)	0.990
	suPAR	1.23 (0.98–1.52)	0.070
	Age	1.05 (1.03–1.07)	< 0.001
	Sex	1.37 (0.91–2.07)	0.131
	Pack-years	1.01 (1.00–1.01)	0.125
	LDL-C	1.00 (1.00–1.01)	0.879
	BPs	1.01 (1.00–1.01)	0.256
T2DM	0.75 (0.51–1.08)	0.124	
	UACR	1.00 (1.00–1.00)	0.015

Estimates of covariates of Cox regression analyses for outcome events for renal parameters.

Multivariable model included adjustment for sex, patient age, LDL-C, systolic blood pressure (BPs), presence of T2DM, and smoking pack-years. eGFR, estimated glomerular filtration rate (according to the Chronic Kidney Disease Epidemiology equation); LDL-C, low-density lipoprotein cholesterol; BPs, systolic blood pressure; suPAR, logarithm (base ten) transformed suPAR level; T2DM, type 2 diabetes; UACR, urine albumin-to-creatinine ratio.

guidelines. Additionally, suPAR was associated with all-cause mortality even after adjustment for classical cardiovascular risk factors and eGFR.

This study showed for the first time an association of suPAR levels and reduced ABI. Restrictively, this finding is only valid for patients without media-sclerosis due to diabetes or CKD.

Elevated suPAR levels have been reported in obese type 2 diabetes without CKD.²⁰ In this study, no elevation of suPAR was delineated due to the diagnosis of type 2

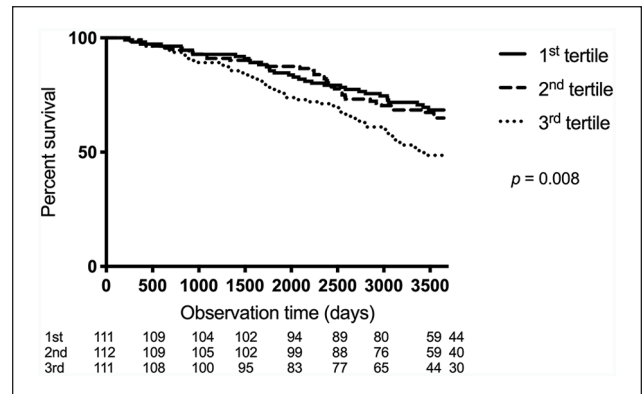


Figure 3. Kaplan–Meier curve for the prediction of all-cause survival according to suPAR tertiles over 10 years. suPAR tertiles were compared using the log-rank test (p -value). suPAR, soluble urokinase-type plasminogen activator receptor.

diabetes mellitus. This discrepancy might be due to good glycemic control in this study (6.8 (6.2–7.5) rel. % vs 7.6 (6.9–9.2) rel. %²⁰). Furthermore, in this PAD study, 78.5% of patients without diabetes were classified as having pre-diabetes. suPAR levels increase in patients with impaired glucose tolerance,³⁶ which might explain the small increase of suPAR levels in patients with type 2 diabetes compared to patients without manifest type 2 diabetes mellitus.

Markers of CKD, such as eGFR and proteinuria, have been associated with elevated suPAR levels.^{17,37} In patients with established atherosclerosis (patients with CAD), suPAR levels were linked to eGFR decline (CKD-EPI equation) and dip-stick proteinuria.¹⁷ Additionally, UACR has been associated with elevated suPAR levels in healthy individuals³⁷ and African American patients with type 2 diabetes mellitus (31% of patients with CAD).³⁸ This study shows the known association of eGFR and suPAR levels and extends the association of UACR and suPAR to a cohort of overt atherosclerosis. Furthermore, we are the first to show a linear increase of suPAR levels from low to very high risk according to the KDIGO CKD guidelines.³⁴ Thus, we might hypothesize that suPAR as a biomarker reflects the possibility of CKD progression in patients with PAD. In this study, in the subgroup of patients with measurable albuminuria below the threshold of 30 mg/g, no significant association of suPAR and UACR was found ($R = 0.017$, $p = 0.791$). This suggests signs of CKD (i.e. measurable albuminuria exist before elevation of suPAR in patients with PAD).

However, due to the study design, we are not able to delineate CKD progression over the study period. Additionally, our cohort represents patients with mild to moderate renal function impairment (eGFR 67.7 (43.9–83.9) mL/min/1.72 m²). In patients with advanced CKD (eGFR 50.9 ± 29.9 mL/min/1.72 m² by Chinese adaptation for modification of diet in renal disease equation), elevated suPAR levels resulted in a higher incidence of dialysis over 6 years.²² However, this Chinese cohort enclosed about 60% glomerulonephritis patients, including patients with focal segmental glomerulosclerosis.²² Categorization of patients with PAD according to KDIGO categories, in addition to traditional risk factors, shows an increased risk for

hospitalization and major adverse limb events in patients with PAD.⁶ Recent research indicates an increase in hospitalization and cardiovascular events in patients with PAD due to CKD.³⁹

In a recent report, elevated suPAR levels were linked to systemic atherosclerosis and an increased mortality risk.⁸ This study mainly included patients suffering from coronary artery disease (CAD) ($n = 3614$) or CAD with additional PAD ($n = 869$) in comparison to 99 patients with PAD alone. PAD was defined as atherosclerosis, including carotid, aortic, subclavian, brachial, iliac, femoral, and popliteal atherosclerosis. Elevated suPAR levels were previously associated with angiographic CAD severity.⁴⁰ Our study defined PAD as lower-limb atherosclerosis ($n = 334$), including 31.7% CAD patients. The current study showed a similar univariate ($p < 0.001$) and multivariable ($p = 0.017$) association with all-cause mortality. This association did sustain further adjustment for CKD (eGFR) but not UACR.

This study shows that suPAR is associated with ABI, renal function, and all-cause mortality. We hypothesize that suPAR primarily reflects the atherosclerotic disease burden (e.g. expression in atherosclerotic lesions) modified by CKD. Thus, the combination of both could affect patients' mortality. The association of suPAR and PAD warrants further research. PAD severity assessed by ABI should be evaluated further in patients with PAD and critical limb ischemia. However, possible wound infections and associated CRP elevation in Fontaine stage IV patients might bias the associations with suPAR. This obstacle could be averted by the inclusion of patients with rest pain (Fontaine stage III) as a comparator. Furthermore, serial suPAR measurements in patients with PAD before and after revascularization procedures could help to delineate whether suPAR reflects the individuals' atherosclerotic burden or is somehow upregulated in states of recurrent hypoxia in the limbs.

Limitations

Several limitations must be considered. First, we evaluated suPAR in stable patients with PAD, thus the association of suPAR and ABI could not be validated in critical limb ischemia. Second, only patients with mild to moderate CKD were included in this study, and our patients ranged from age 66 ± 10 to 72 ± 11 in years in the lowest and highest suPAR tertile, respectively. Therefore, using the CKD-EPI equation for eGFR might result in overestimated filtration rates in elderly patients. Unfortunately, we did not measure cystatin C, allowing us to use eGFR formulas known to be more accurate in elderly people. However, we calculated our statistics using the creatinine-based Berlin Initiative Study 1 (BIS1) formula, which is described to be an acceptable alternative if cystatin C is not available.⁴¹ Comparing our results of the CKD-EPI formula and the BIS1 formula, no significant differences were observed. suPAR levels, according to eGFR using the BIS1 formula are shown in online supplemental figure 2. Third, the association with all-cause mortality remains hypothesis-generating and can only suggest, but not prove, confounding by CKD in an observational trial.

Conclusion

In summary, we were able to demonstrate an association between PAD severity and suPAR levels. Additionally, suPAR levels were associated with declined eGFR and elevated UACR in patients with overt atherosclerosis. Higher levels of suPAR were associated with increased all-cause mortality. Further research of suPAR in patients with PAD to extend the current knowledge into critical limb ischemia is warranted.

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Supplementary material

The supplementary material is available online with the article.

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