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Circulating soluble urokinase plasminogen activator receptor levels and peripheral arterial disease outcomes

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Abstract

\textbf{Background and aims—}Circulating soluble urokinase plasminogen activator receptor (suPAR) is a marker of immune activation associated with atherosclerosis. Whether suPAR levels are associated with prevalent peripheral arterial disease (PAD) and its adverse outcomes remains unknown and is the aim of the study.

\textbf{Methods—}SuPAR levels were measured in 5810 patients (mean age 63 years, 63\% male, 77\% with obstructive coronary artery disease [CAD]) undergoing cardiac catheterization. The presence of PAD (n = 967, 17\%) was classified as carotid (36\%), lower/upper extremities (30\%), aortic (15\%) and multisite disease (19\%). Multivariable logistic and Cox regression models were used to determine independent predictors of prevalent PAD and outcomes including all-cause death, cardiovascular death and PAD-related events after adjustment for age, gender, race, body mass index, smoking, diabetes, hypertension, hyperlipidemia, renal function, heart failure history, and obstructive CAD.

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\textbf{Conflict of interest}
The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

\textbf{Author contributions}
Analysis and interpretation of data: Ayman Samman Tahhan, Yi-An Ko.
Drafting of manuscript: Ayman Samman Tahhan, Jamal Hajjari, Arshed Quyyumi.
Critical revision: Shipra Arya, Jochen Reiser, Viola Vaccarino, Laurence Sperling.
Results—Plasma suPAR levels were 22.5% ($p < 0.001$) higher in patients with PAD compared to those without PAD. Plasma suPAR was higher in patients with more extensive PAD ($\geq 2$ compared to single site) $p < 0.001$. After multivariable adjustment, suPAR was associated with prevalent PAD; odds ratio (OR) for highest compared to lowest tertile of 2.0, 95% CI (1.6–2.5) $p < 0.001$. In Cox survival analyses adjusted for clinical characteristics and medication regimen, suPAR (in the highest vs. lowest tertile) remained an independent predictor of all-cause death [HR 3.1, 95% CI (1.9–5.3)], cardiovascular death [HR 3.5, 95% CI (1.8–7.0)] and PAD-related events [HR = 1.8, 95% CI (1.3–2.6) $p < 0.001$ for all].

Conclusions—Plasma suPAR level is predictive of prevalent PAD and of incident cardiovascular and PAD-related events. Whether SuPAR measurement can help screen, risk stratify, or monitor therapeutic responses in PAD requires further investigation.

Keywords
SuPAR; PAD; CAD; Atherosclerosis; Cardiovascular outcomes; PAD-Related outcomes

1. Introduction

Atherosclerosis is a systemic disease that can involve coronary, cerebrovascular, aortic, renal, upper and lower extremity arteries [1,2]. Although widely prevalent, peripheral artery disease (PAD) remains underdiagnosed and is associated with excessive morbidity and mortality [3]. Despite sharing common risk factors with coronary artery disease (CAD) including hypertension, advanced age, diabetes, hyperlipidemia and smoking, only 20–30% of patients with CAD develop concomitant significant PAD. In addition, endothelial dysfunction, reduced regenerative capacity, inflammation and immune dysregulation also play a fundamental role in the development and progression of PAD [1,2,4–6]. However, none of the markers reflecting these processes have been translated into clinical practice either for screening, risk stratification, or to monitor therapeutic response in patients with PAD.

Recently, we and others have shown that higher levels of soluble urokinase plasminogen activator receptor (suPAR), a circulating marker of inflammation, thromobogenesis, and immune regulation, are associated with hypertension, diabetes, CAD, stroke, and chronic kidney disease [7–10]. SuPAR is produced by cleavage of membrane-bound urokinase-type plasminogen activator receptor, which is a membrane linked G-protein expressed on numerous inflammatory cells including monocytes, activated T-lymphocytes and macrophages as well as fibroblasts and endothelial cells. Both the circulating and membrane-bound forms are directly involved in the regulation of cell adhesion and migration through binding of integrins. The soluble form has direct chemotactic properties that may facilitate recruitment of inflammatory cells such as neutrophils and monocytes and the mobilization of hematopoietic stem cells [11–13]. Finally, SuPAR is associated with increased inflammatory activity in atherosclerotic plaques [10].

The relationship between circulating suPAR levels and PAD and its related outcomes has not been systematically studied to date. Whether suPAR levels predict presence of PAD and whether its’ levels are a measure of long-term incident risk remains unknown. The aim of
this study is to examine the association between plasma suPAR levels and the presence of PAD and adverse cardiovascular and PAD-related events.

2. Materials and methods

2.1. Study design and population

We measured suPAR levels in 5810 adults ≥18 years from the Emory Cardiovascular Biobank, a prospective cohort of patients undergoing left heart catheterization for suspected or confirmed CAD at Emory Healthcare sites in Atlanta, GA, between 2003 and 2015. Subjects with congenital heart disease, heart transplantation, severe anemia, active inflammatory diseases, and cancer were excluded. Participants were interviewed to collect demographic characteristics, medical history, medication use, and behavioral habits as previously described [14]. Medical records and ICD-9 diagnostic codes were reviewed to confirm self-reported medical history. Angiograms performed at enrollment were reviewed, and obstructive CAD was defined as the presence of ≥50% stenosis in at least one coronary artery. The study was approved by the institutional review board at Emory University (Atlanta, GA). All subjects provided written informed consent at the time of enrollment.

2.2. Defining peripheral arterial disease

We extensively reviewed patients’ self-reported and physician-documented medical history and imaging reports to identify the presence of PAD as previously described [6]. PAD was defined as a history of symptomatic or asymptomatic non-coronary atherosclerotic disease in at least one of the following arteries: carotid, thoracic or abdominal aorta, subclavian, brachial, iliac, femoral, or popliteal arteries. No routine testing was performed to screen for asymptomatic PAD. PAD of the lower extremities was diagnosed when at least one of the following were present: documented ankle-brachial index <0.90; lower limb revascularization; atherosclerotic plaques or stenosis on imaging ≥50% (computed tomography, ultrasound, or fluoroscopy) in the iliac, femoral, or popliteal arteries; and history of amputation for critical limb ischemia. All subjects with extremities PAD were symptomatic. PAD of the carotid artery was diagnosed if there was ≥20% stenosis in any carotid artery. Subgroup analysis was done to compare those with mild vs. severe carotid disease using higher cutoffs of 25% and 50%. Aortic disease was diagnosed when there was a history abdominal (greater than 4 cm) or thoracic (ascending greater than 4 cm or descending diameter larger than 3 cm) aneurysms after (excluding subjects with aortic root aneurysm associated with bicuspid aortic valves) or evidence of moderate-to-severe (frequent, large plaques) atherosclerotic plaques of the aorta or renal arteries on computed tomography imaging.

2.3. Sample collection and measurement of suPAR

Fasting arterial blood samples were collected at the time of catheterization and stored at −80 °C. Plasma levels of suPAR were measured (suPARnostic kit; ViroGates, Copenhagen, Denmark) with a lower detection limit of 100 pg/mL and intra- and inter-assay variation of 2.75% and 9.17%, respectively [8]. Serum high-sensitivity C-reactive protein (CRP) levels were determined in 3452 patients (59.4%) using a particle-enhanced immunoturbidimetry...
assay (FirstMark, a division of GenWay Biotech) that has a lower limit of detection of 0.03 mg per liter [15].

2.4. Follow-up and outcomes

We conducted follow-up as previously described to identify prespecified incident adverse cardiovascular outcomes including death, cardiovascular death and PAD-related events. In brief, follow-up and adjudication were conducted by personnel blinded to the SuPAR data by phone, electronic medical record review, as well as and social security death index and state records. PAD-related events such as peripheral revascularization, amputation and stroke were identified using standard current procedural terminology codes for vascular procedures, chart review and followup questionnaire [6].

2.5. Statistical analysis

We reported subject characteristics as descriptive statistics with means, standard deviations, frequency counts, percentages, medians, and interquartile ranges. Differences between groups were assessed using the t-test for continuous variables, and chi-square for categorical variables. Two-tailed P-value ≤0.05 were considered statistically significant. For non-normally distributed variables such as suPAR and CRP levels, the Mann-Whitney U test was used to compare groups in unadjusted analyses. For multivariable analyses, suPAR levels were examined both as a categorical variable stratified by tertiles, and as a continuous variable after log-transforming, and reported as “per 25% increase in suPAR”. Covariates incorporated in multivariable analyses included age, gender, race, body mass index (BMI), smoking history, hypertension, diabetes, estimated glomerular filtration rate (eGFR calculated using the CKD-EPI equation), presence of obstructive CAD, statin use, antiplatelet therapy, angiotensin pathway antagonist use, beta-blocker therapy, suPAR and hs-CRP levels. Logistic regression was performed to investigate independent predictors of prevalent PAD. Regression coefficients are presented as point estimates with 95% confidence intervals. We examined the collinearity among all the continuous variables using Pearson and Spearman correlation when appropriate. We found that the highest two correlations observed were 0.4 and 0.5. We further examined the tolerance values and none of them were <0.1. We also did not find a large standard errors for any of the estimates. The Kaplan-Meier curves as well as Cox proportional-hazards regression model were used to examine the association between suPAR and all-cause death, cardiovascular death, and PAD-related outcomes. Analysis was conducted using available data (9% with missing data) under the assumption of missing completely at random. Analyses were performed using IBM SPSS Statistics Version 22, (Armonk, NY, USA) and R 3.2.2 (R Core Team, Vienna, Austria). Lastly, we examined the incremental value of adding suPAR levels to a clinical model for predicting death. The c-statistic, continuous net reclassification improvement (NRI), and integrated discrimination improvement (IDI) were calculated to evaluate the improvement in predictive ability of the models with and without SuPAR using the R packages survC1 and survIDINRI.
3. Results

Characteristics of the 5810 patients enrolled are shown in Table 1. The mean age of the cohort was 63.5 (±12.2) years, 63% male and 77% had significant CAD (>50% luminal stenosis).

3.1. SuPAR and PAD characteristics

Baseline characteristics according to CAD and PAD diagnoses are shown in Table 1 and Supplemental Table 1. The 967 (17%) patients with PAD were more likely to be older and have more cardiovascular risk factors, worse renal function, and were more likely to have CAD. Presence of PAD (n = 967, 17%) was classified as carotid (36%), lower/upper extremities (30%), aortic (15%) and multisite disease (19%). Most subjects with multisite disease (74%) have extremities PAD. Overall, 43% of subjects with PAD had atherosclerosis involvement in upper or lower extremities. The distribution of extremities PAD is even higher (54.5%) after excluding those with mild-to-moderate carotid disease with stenosis <50%. Among subjects with PAD in the extremities, 303 (93%) were in the lower and only 20 (7%) in the upper extremities.

Plasma suPAR levels were 22.5% higher [median of 3474 interquartile range IQR (2691–4653)] in patients with PAD compared to those without [2836 pg/mL IQR (2230–3739), p < 0.001]. Plasma suPAR levels were highest in patients with CAD and PAD followed by PAD alone, CAD alone and no CAD or PAD groups, p < 0.001 (Table 1 and Fig. 1A) and highest in those with more extensive PAD (≥ 2 sites) compared to single site PAD, p < 0.001 (Fig. 1B). Moreover, levels of suPAR were highest in patients with multisite PAD [median of 3695 IQR (2717–5167)] followed by upper or lower extremities PAD [3641 IQR (2855–4735)], carotid [3550 IQR (2766–4828)], and aorta [3234 IQR (2396–4026)] p = 0.03 for comparison. SuPAR levels were similar in those with mild vs. severe carotid stenosis using 25% or even 50% stenosis as cutoff (p > 0.4). SuPAR levels were slightly higher in individuals with symptomatic [3432 IQR (2621–4593)] vs. asymptomatic [3508 IQR (2760–4719)] PAD but didn’t reach statistical significance (p = 0.2).

In multivariable analyses, independent predictors for the presence of PAD included age, smoking, BMI, diabetes, hypertension, eGFR, heart failure, obstructive CAD and plasma suPAR level (Table 2). SuPAR in the highest compared to lowest tertile had an odds ratio (OR) of 2.0 (95% CI 1.6–2.5) p < 0.001 for presence of PAD. Similarly, using suPAR as a continuous variable, for each 25% increase in suPAR level, the OR of prevalent PAD increased by 13% (95% CI 1.08–1.18) p < 0.001 (Table 2).

In the 3452 (59.4%) patients with hs-CRP levels, there was no association between hs-CRP levels and prevalent PAD. Moreover, suPAR remained an independent predictor of PAD after adjustment for the hs-CRP level.

3.2. SuPAR and cardiovascular outcomes in PAD

In 968 patients with PAD, there were 245 (25.3%) deaths from all causes, 156 (16.1%) deaths from cardiovascular causes, and 79 (8.2%) incident myocardial infarcts over a median follow-up period of 3.4 (IQR 1.6–6.6) years. In unadjusted analyses, a 25% increase in
suPAR level was associated with increased risk of all-cause death [HR = 1.4 (95% CI 1.3–1.4) p < 0.001] and cardiovascular death [HR = 1.4, (95% CI 1.3–1.5), p < 0.001]. Kaplan–Meier survival curves for association between suPAR tertiles and clinical outcomes are shown in Fig. 2. Subjects in the highest compared to lowest suPAR tertiles had higher all-cause (40.7% vs. 10.8%) and cardiovascular death rates (25.5% vs. 5.9%). After adjusting for demographics, clinical characteristics and medication regimen as detailed above, plasma suPAR level (highest compared to the lowest tertile) remained an independent predictor of all-cause death (HR 3.1, 95% confidence interval CI [1.9–5.3]), cardiovascular death (HR 3.5, 95% CI [1.8–7.0]) and combined cardiovascular death/MI (HR 2.6, 95% CI [1.5–4.7]) (p < 0.001 for all outcomes), Table 3. We obtained similar results using a model adjusted for variables that correlated in univariate analysis with the outcome of death (included black, age, BMI, heart failure, diabetes, statin use, and eGFR). These results remained significant even after adjusting for hs-CRP levels (n = 524).

Mortality was higher in subjects with multisite PAD (34.6%) followed by extremities PAD (32.1%) followed by aortic PAD (28.2%) followed by carotid PAD (26.0%) although this didn’t reach statistical significance (Log Rank p = 0.32). Death rate was similar in those with mild vs. severe carotid stenosis using 50% as cutoff (25% vs 28%, p = 0.4). Subjects with symptomatic PAD had increased risk of death compared to asymptomatic subjects with HR 1.29 (95% CI 1.03–1.63); p = 0.04 but became not significant in fully adjusted model (p = 0.096).

In patients with and without PAD, there were 347 (6.6%) PAD-related events over the follow-up period. In an adjusted Cox regression model, independent predictors of PAD-related events were smoking, lower BMI, hypertension, CAD, HF, clopidogrel use, and the suPAR level [HR = 1.8, 95% CI (1.4–2.5) p < 0.001] highest vs. lowest tertile. Similarly, each 25% increase in the suPAR level was associated with a 17% increase in the risk of PAD-related events [HR = 1.17 95% CI (1.09–1.24) p < 0.001], Table 4 and Fig. 3.

We tested the incremental value of adding suPAR as a continuous variable to a model with significant traditional risk factors and clinical characteristics in predicting PAD-related outcomes. Addition of suPAR improved the C-statistic for PAD-related outcomes (from 0.704 to 0.720 Δ = 0.0152, [95% CI –0.003–0.034]), the continuous net reclassification improvement (0.19 [95% CI 0.09–0.26], p = 0.004) and integrated discrimination improvement (0.007 [95% CI 0.002 0.019], p < 0.001).

4. Discussion

This is the first study to demonstrate that elevated plasma SuPAR levels are associated with prevalent PAD and its adverse outcomes. SuPAR levels are higher in subjects with PAD compared to those with only CAD and are highest in those with both PAD and CAD. Furthermore, patients with more diffuse and multi-site PAD have the highest levels of SuPAR. Most importantly, a higher SuPAR level was associated with an increase in all-cause and cardiovascular mortality, as well as PAD-related events and improved risk discrimination metrics when added to a model with traditional risk factors.
We and others have previously reported that elevated suPAR levels are associated with cardiovascular risk factors, subclinical and clinical cardiovascular disease including CAD, adverse cardiovascular outcomes and progression of chronic kidney disease [7,16-18,8,9,19-26]. Higher plasma and intra-plaque suPAR levels were noted to be associated with symptomatic carotid stenosis and vulnerable plaques [10]. Another study showed that uPAR expression in the intima of atherosclerotic lesions was progressively higher with increasing severity of atherosclerosis in human coronary and aortic vessel segments [27]. Our current investigation extends these findings to subjects with PAD. We also found a graded relationship between the extent of peripheral atherosclerosis, estimated as the number of diseased vascular beds and suPAR levels, suggesting that suPAR levels reflect the underlying atherosclerotic burden whether it involves the coronary or peripheral circulations.

Atherosclerosis is a complex process that is initiated by injury to the vascular endothelium, formation of plaque and its subsequent expansion and often sudden progression to acute coronary syndromes [1]. Endothelial injury promotes activation and migration of inflammatory cells into the sub-endothelial space with differentiation into macrophages [1,28], processes that are modulated by uPAR and suPAR. Experimental studies have demonstrated that uPAR and its soluble form, suPAR play a critical role in cell adhesion and migration, intercellular signaling, matrix degradation and proliferation [18,28–30]. suPAR can downregulate immune defenses by inhibiting neutrophil efferocytosis (clearance of apoptotic cells) and membrane-bound uPAR facilitates phagocytosis.

The role of inflammatory biomarkers in PAD has been previously studied. For example, interleukin-6, tumor necrosis factor α, intercellular adhesion molecules and CRP levels are higher in patients with PAD [31–33] but their role on predicting PAD-related events remains unclear as these follow-up studies are small and unvalidated [34]. In almost 1000 patients extensively phenotyped for PAD, we demonstrate the value of suPAR in predicting incident adverse events independent of hs-CRP levels, suggesting that suPAR reflects more than an activated inflammatory state.

Our study has important strengths. These include a large cohort size with detailed phenotyping of PAD, long term follow-up with a large number of cardiovascular and PAD-related events, and exploration of the interaction with inflammation assessed by hs-CRP. It is possible that some patients with asymptomatic PAD were undiagnosed because of the lack of systematic screening that remains a limitation of our cohort.

In conclusion, this is the first and largest comprehensive study to demonstrate that levels of suPAR are higher in patients with PAD, particularly in those with extensive atherosclerosis and are predictive of long term cardiovascular and PAD-related outcomes. Whether SuPAR is a useful biomarker to screen for, risk stratify, or to monitor therapeutic response to PAD treatment requires further investigation.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2017.06.019.

References


Fig. 1.
Boxplots.
(A) Boxplot showing suPAR levels among patients with no CAD or PAD compared to those with known CAD, PAD, and PAD and CAD. *p* value for trend. (B) Boxplot showing suPAR levels among patients with no PAD compared to those with PAD at one site and PAD at ≥2 sites. Body extends from the first to third quartile, horizontal line in the box represents the median, whiskers represent 5% and 95% values.
Fig. 2.
Kaplan–Meier curves for association between levels of suPAR by tertiles for the primary end point of (A) all-cause death (B) cardiovascular death and (C) combined cardiovascular death/myocardial infarction.
Fig. 3.
Kaplan–Meier curves for association between levels of suPAR by tertiles for PAD-related events.
Blue line represents suPAR in the lowest tertile, green line represents suPAR in the middle tertile and red line represents suPAR in the highest tertile. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Table 1
Baseline characteristics among patients with and without PAD.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 5810)</th>
<th>No PAD (n = 4842)</th>
<th>PAD (n = 968)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years mean (SD)</td>
<td>63.5 (12.2)</td>
<td>62.6 (12.1)</td>
<td>68 (11.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male n (%)</td>
<td>3701 (63.2)</td>
<td>3040 (62.8)</td>
<td>631 (65.2)</td>
<td>0.16</td>
</tr>
<tr>
<td>Black n (%)</td>
<td>1362 (23.3)</td>
<td>1119 (23.1)</td>
<td>209 (21.6)</td>
<td>0.30</td>
</tr>
<tr>
<td>Body Mass Index kg/m(^2) mean (SD)</td>
<td>29.8 (6.4)</td>
<td>30 (6.4)</td>
<td>28.7 (6.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estimated GFR mL/min/1.73 m(^2) mean (SD)</td>
<td>73 (23.9)</td>
<td>74.8 (23.2)</td>
<td>63.5 (25.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking n (%)</td>
<td>3821 (65.2)</td>
<td>3074 (63.5)</td>
<td>722 (74.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes n (%)</td>
<td>2030 (35.4)</td>
<td>1603 (33.6)</td>
<td>420 (44.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>4578 (79)</td>
<td>3711 (77)</td>
<td>854 (88.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypercholesterolemia n (%)</td>
<td>4135 (71.6)</td>
<td>3372 (70.1)</td>
<td>753 (78.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acute myocardial infarction n (%)</td>
<td>507 (8.7)</td>
<td>418 (8.6)</td>
<td>89 (9.2)</td>
<td>0.57</td>
</tr>
<tr>
<td>Obstructive CAD n (%)</td>
<td>4498 (76.8)</td>
<td>3614 (74.6)</td>
<td>869 (89.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of heart failure n (%)</td>
<td>1917 (32.7)</td>
<td>1463 (30.2)</td>
<td>419 (43.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ejection fraction% mean (SD)</td>
<td>52.8 (12.7)</td>
<td>53.2 (12.5)</td>
<td>50.7 (13.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of myocardial infarction n (%)</td>
<td>1378 (24.5)</td>
<td>1050 (22.4)</td>
<td>325 (35.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of PCI n (%)</td>
<td>2610 (44.6)</td>
<td>2053 (42.4)</td>
<td>549 (56.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of CABG n (%)</td>
<td>1416 (24.2)</td>
<td>1017 (21)</td>
<td>395 (40.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACE/ARB use n (%)</td>
<td>3164 (54)</td>
<td>2570 (53.1)</td>
<td>574 (59.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aspirin use n (%)</td>
<td>4292 (73.3)</td>
<td>3464 (71.5)</td>
<td>798 (82.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clopidogrel use n (%)</td>
<td>2543 (43.4)</td>
<td>1999 (41.3)</td>
<td>535 (55.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statin use n (%)</td>
<td>4011 (68.5)</td>
<td>3214 (66.4)</td>
<td>768 (79.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beta blocker use n (%)</td>
<td>3840 (65.6)</td>
<td>3067 (63.3)</td>
<td>743 (76.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hs-CRP (mg/dL) median (IQR)</td>
<td>2.9 (1.2–7.5)</td>
<td>2.9 (1.1–7.3)</td>
<td>3.1 (1.4–8.5)</td>
<td>0.02</td>
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<tr>
<td>SuPAR (pg/mL) median (IQR)</td>
<td>2944 (2285.81–3925)</td>
<td>2836 (2230–3739)</td>
<td>3474 (2691–4653)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* p-value compares patients with and without PAD.
Table 2

Multivariable analysis using logistic regression for predictors of prevalent PAD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>Lower CI</th>
<th>Upper CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02</td>
<td>1.01</td>
<td>1.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>1.07</td>
<td>0.91</td>
<td>1.26</td>
<td>0.40</td>
</tr>
<tr>
<td>Black</td>
<td>1.09</td>
<td>0.90</td>
<td>1.33</td>
<td>0.37</td>
</tr>
<tr>
<td>Smoking history</td>
<td>1.65</td>
<td>1.39</td>
<td>1.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>0.97</td>
<td>0.96</td>
<td>0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.26</td>
<td>1.07</td>
<td>1.48</td>
<td>0.006</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.57</td>
<td>1.24</td>
<td>1.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>1.12</td>
<td>0.92</td>
<td>1.35</td>
<td>0.26</td>
</tr>
<tr>
<td>Estimated GFR</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obstructive CAD</td>
<td>1.99</td>
<td>1.56</td>
<td>2.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart failure history</td>
<td>1.38</td>
<td>1.18</td>
<td>1.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SuPAR above median</td>
<td>1.69</td>
<td>1.43</td>
<td>2.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SuPAR lowest tertile</td>
<td>1.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SuPAR middle tertile</td>
<td>1.42</td>
<td>1.15</td>
<td>1.74</td>
<td>0.00</td>
</tr>
<tr>
<td>SuPAR highest tertile</td>
<td>2.02</td>
<td>1.63</td>
<td>2.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SuPAR 25% increase</td>
<td>1.13</td>
<td>1.08</td>
<td>1.18</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3

Adjusted Cox regression model for the association between suPAR level and adverse outcomes in patients with PAD (n = 968).

| Death (n = 245) | SuPAR (25% increase) | 1.34 | 1.23 | 1.45 | <0.001 |
| SuPAR middle vs. lowest tertile | 1.87 | 1.09 | 3.21 | 0.02 |
| SuPAR highest vs. lowest tertile | 3.15 | 1.88 | 5.27 | <0.001 |

| Death (n = 245) | SuPAR (25% increase) | 1.30 | 1.18 | 1.44 | <0.001 |
| SuPAR middle vs. lowest tertile | 2.42 | 1.20 | 4.88 | 0.01 |
| SuPAR highest vs. lowest tertile | 3.53 | 1.78 | 6.97 | 0.0003 |

| CV death (n = 156) | SuPAR (25% increase) | 1.22 | 1.11 | 1.33 | <0.001 |
| SuPAR middle vs. lowest tertile | 1.92 | 1.07 | 3.44 | 0.03 |
| SuPAR highest vs. lowest tertile | 2.65 | 1.50 | 4.68 | 0.001 |

Adjusted Cox regression model for age, gender, race, body mass index, smoking, diabetes mellitus, hypertension, hypercholesterolemia, eGFR, CAD, and medication use including aspirin, statins, beta-blockers, ACE inhibitors/ARB, and clopidogrel.
Table 4

Adjusted Cox regression model for the association between suPAR level and PAD-related outcomes (n = 5217, 347 events).

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>lower 95% CI</th>
<th>Upper 95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking history</td>
<td>1.27</td>
<td>1.00</td>
<td>1.61</td>
<td>0.049</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.38</td>
<td>1.63</td>
<td>3.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (1 unit increase)</td>
<td>0.97</td>
<td>0.95</td>
<td>0.99</td>
<td>0.002</td>
</tr>
<tr>
<td>Obstructive CAD</td>
<td>2.12</td>
<td>1.41</td>
<td>3.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart failure history</td>
<td>1.38</td>
<td>1.11</td>
<td>1.72</td>
<td>0.004</td>
</tr>
<tr>
<td>Clopidogrel use</td>
<td>1.46</td>
<td>1.14</td>
<td>1.87</td>
<td>0.003</td>
</tr>
<tr>
<td>SuPAR middle vs. lowest tertile</td>
<td>1.40</td>
<td>1.05</td>
<td>1.88</td>
<td>0.023</td>
</tr>
<tr>
<td>SuPAR highest vs. lowest tertile</td>
<td>1.84</td>
<td>1.36</td>
<td>2.51</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Adjusted Cox regression model for age, gender, race, body mass index, smoking, diabetes mellitus, hypertension, hypercholesterolemia, eGFR, CAD, and medication use including aspirin, statins, beta-blockers, ACE inhibitors/ARB, and clopidogrel.