

# Predicting Mortality in African Americans With Type 2 Diabetes Mellitus: Soluble Urokinase Plasminogen Activator Receptor, Coronary Artery Calcium, and High-Sensitivity C-Reactive Protein

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**Background**—Type 2 diabetes mellitus is a major risk factor for cardiovascular disease; however, outcomes in individual patients vary. Soluble urokinase plasminogen activator receptor (suPAR) is a bone marrow–derived signaling molecule associated with adverse cardiovascular and renal outcomes in many populations. We characterized the determinants of suPAR in African Americans with type 2 diabetes mellitus and assessed whether levels were useful for predicting mortality beyond clinical characteristics, coronary artery calcium (CAC), and high-sensitivity C-reactive protein (hs-CRP).

**Methods and Results**—We measured plasma suPAR levels in 500 African Americans with type 2 diabetes mellitus enrolled in the African American-Diabetes Heart Study. We used Kaplan-Meier curves and Cox proportional hazards models adjusting for clinical characteristics, CAC, and hs-CRP to examine the association between suPAR and all-cause mortality. Last, we report the change in C-statistics comparing the additive values of suPAR, hs-CRP, and CAC to clinical models for prediction of mortality. The suPAR levels were independently associated with female sex, smoking, insulin use, decreased kidney function, albuminuria, and CAC. After a median 6.8-year follow-up, a total of 68 deaths (13.6%) were recorded. In a model incorporating suPAR, CAC, and hs-CRP, only suPAR was significantly associated with mortality (hazard ratio 2.66, 95% confidence interval 1.63–4.34). Addition of suPAR to a baseline clinical model significantly improved the C-statistic for all-cause death ( $\Delta 0.05$ , 95% confidence interval 0.01–0.10), whereas addition of CAC or hs-CRP did not.

**Conclusions**—In African Americans with type 2 diabetes mellitus, suPAR was strongly associated with mortality and improved risk discrimination metrics beyond traditional risk factors, CAC and hs-CRP. Studies addressing the clinical usefulness of measuring suPAR concentrations are warranted. (*J Am Heart Assoc.* 2018;7:e008194. DOI: 10.1161/JAHA.117.008194.)

**Key Words:** biomarker • calcium score • soluble urokinase plasminogen activator receptor • urokinase

Type 2 diabetes mellitus (T2D) is a major risk factor for cardiovascular and kidney disease. It is estimated to affect more than 350 million adults worldwide, and its

incidence continues to increase.<sup>1</sup> Guidelines for the assessment and management of cardiovascular risk consider a diagnosis of T2D equivalent to coronary artery disease (CAD).<sup>2,3</sup> However, outcomes are heterogeneous, with several recent studies suggesting that the risk of CAD in T2D is less than that in patients with preexisting CAD and that the overall incidence of CAD in patients with diabetes mellitus may be lower than previously reported.<sup>4,5</sup> Both high-sensitivity C-reactive protein (hs-CRP) and cardiac imaging, specifically coronary artery calcium (CAC), have been proposed as tools to improve risk stratification in patients with T2D, perhaps allowing for the identification of a subgroup for which statin therapy could be deferred; however, their prognostic and clinical utility have been questioned.<sup>6–8</sup>

Soluble urokinase plasminogen activator receptor (suPAR) is a marker of immune activation thought to be involved in the pathogenesis of kidney disease; suPAR concentrations have also been associated with mortality and cardiovascular disease in the general population (without comorbidities), in

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## Clinical Perspective

### What Is New?

- This study compared soluble urokinase plasminogen activator receptor (suPAR) to C-reactive protein and coronary artery calcium in predicting death in African Americans with type 2 diabetes mellitus.
- Patients with type 2 diabetes mellitus on insulin therapy had significantly higher suPAR levels compared to those not using insulin.
- suPAR levels were strongly associated with all-cause death above and beyond traditional risk factors as well as C-reactive protein and coronary artery calcium.

### What Are the Clinical Implications?

- SuPAR levels may be a surrogate marker for the underlying inflammatory state associated with insulin resistance.
- As a biomarker of risk for kidney disease and all-cause death, suPAR levels may be useful in patients with type 2 diabetes mellitus for risk stratification, initiation of preventive therapy such as statins, renin-angiotensin inhibitors, or treatment decisions such as in coronary artery bypass grafting versus percutaneous coronary intervention in patients with multivessel disease.

critically ill patients, patients with cerebrovascular disease, HIV infection, and kidney disease.<sup>9-21</sup> In contrast to hs-CRP, elevated suPAR is reportedly associated with the presence of CAC as well as peripheral arterial disease.<sup>22,23</sup> Whether suPAR levels provide further benefit in risk stratification in African Americans (AA) with T2D has not been studied. We sought to characterize determinants of suPAR levels in patients with T2D and to ascertain whether suPAR is a predictor of CAC and whether its levels are useful for predicting mortality beyond conventional clinical characteristics, CAC, and hs-CRP.

## Methods

### Study Design and Population

We measured plasma suPAR levels in 500 self-reported adult AAs with T2D from the AA-DHS (African American-Diabetes Heart Study). As reported, recruitment came from medical clinics and community advertising.<sup>24,25</sup> Participants underwent physical examinations and were interviewed to collect demographic characteristics, medical history, medication use, and behavioral habits in the Wake Forest School of Medicine Clinical Research Unit.<sup>25</sup> Fasting blood and urine samples were collected at enrollment. Participants with a known serum creatinine concentration  $\geq 2$  mg/dL or physician-diagnosed kidney disease were excluded. T2D was defined

as a diagnosis after the age of 30 years and receiving blood sugar-lowering medications in the absence of diabetic ketoacidosis or insulin-only treatment since diagnosis.

The Chronic Kidney Disease Epidemiology Collaboration equation was used to compute estimated glomerular filtration rate (eGFR).<sup>26</sup> All-cause death was determined by querying the National Death Index, which is an annually updated database maintained by the Centers for Disease Control's National Center for Health Statistics that contains demographic and vital status data for deaths occurring after 1978.<sup>27</sup> The study was approved by the Wake Forest School of Medicine Institutional Review Board (Winston-Salem, NC). All subjects provided written informed consent at the time of enrollment. The data, analytic methods, and study materials can be made available to other researchers for purposes of reproducing the results or replicating the procedure on request.

### Sample Collection and Measurement of suPAR and hs-CRP

Fasting venous blood was collected and stored at  $-80^{\circ}\text{C}$  (never thawed) for a mean duration of 4.9 years before assay. Plasma levels of suPAR were measured using human uPAR Quantikine ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instruction. The minimum detectable suPAR concentration is 33 pg/mL. The intra-assay coefficients of variation for low (mean 836 pg/mL), medium (1593 pg/mL), and high (2412 pg/mL) suPAR levels were 2.1%, 4.1%, and 7.5%, respectively. Human serum hs-CRP was measured by the Hypertension Core Lab, Wake Forest University Health Sciences, using high-sensitivity Enzyme Immuno Assay (ALPCo, Salem, NH), a sandwich assay for determination of CRP in serum, plasma, and urine.

### Arterial Calcium Scoring

As reported, participants were placed in the supine position on the computed tomography couch over a calibration phantom with verified concentrations of calcium hydroxyl apatite (Image Analysis, Inc, Columbia, KY) for scans of the thorax and abdomen.<sup>28</sup> Coronary imaging was performed without intravenous contrast and with electrocardiographic gating in late diastole (75% of the RR interval). Series through the neck for the carotid bifurcation and abdomen for the aortoiliac arteries were performed without electrocardiographic gating using helical scan mode. Computed tomography scans of the coronary, carotid, and aortoiliac vascular territories were analyzed on a GE Advantage Windows Workstation with the SmartScores software package (GE Healthcare, Waukesha, WI) using a modified Agatston scoring method that adjusts for slice thickness and uses the

conventional threshold of 130 Hounsfield units as well as a calcium mass score using a 90 Hounsfield unit threshold. The calcium mass score was employed to have a more stable measure of calcified plaque across the 3 vascular beds. The coronary calcium mass score was the sum of calcium plaque score in epicardial coronary arteries (left main, anterior descending, circumflex, right, and posterior descending). The carotid calcium plaque mass score was the sum of plaque in the common and internal carotid arteries. The aortoiliac calcium plaque mass was the sum of calcium plaque present in the abdominal aorta below the renal arteries and in the right and left common iliac arteries. Arterial segments identified as having stents were defined as artifacts and excluded from quantification, as was CAC in participants who had undergone coronary artery bypass grafting and carotid artery calcium in those who underwent carotid endarterectomy.

## Statistical Analysis

We reported subject characteristics as descriptive statistics with means, medians, standard deviations, and ranges. Between-group differences were assessed using the *t* test for continuous variables, and chi-squared or Fisher exact tests for categorical variables where appropriate. For nonnormally distributed variables such as suPAR, hs-CRP, and CAC levels, the Mann-Whitney U test was used to compare groups in unadjusted analyses. We correlated baseline suPAR levels with enrollment CAC levels ( $n=500$ ) and a subset of 271 patients with 5-year follow-up using the Spearman-Rank test.

We used principal component analysis to derive an estimate of the total burden of calcified atherosclerotic plaque using a linear combination of coronary, carotid, and aortic calcium plaque scores. Each calcium plaque score was standardized by subtracting its median and dividing the observed difference by the square of the interquartile range. The correlation matrix used in the principal component analysis was computed using the standardized scores to account for the differences in measurement scales among the 3 variables. The first principal component explained 83% of the total variation observed in the aforementioned 3 vascular beds and was computed as principal component 1 =  $0.19 \times \text{aorta} + 0.95 \times \text{carotid} + 0.25 \times \text{CAC}$ .

We used multivariable linear regression adjusting for age, sex, body mass index, smoking history, hypertension, eGFR, urine albumin-creatinine ratio, statin use, high- and low-density lipoprotein levels, hemoglobin A<sub>1c</sub>, insulin treatment, duration of T2D, hs-CRP levels, and CAC to identify measures and characteristics independently associated with suPAR levels expressed as per 100% increase in suPAR after base-2 log-transformation. We examined the association between suPAR and all-cause mortality using Cox regression analyses

adjusting for the aforementioned variables in a stepwise fashion, with model 1 incorporating clinical characteristics and hs-CRP, followed by model 2 in which suPAR was added, and, last, model 3 incorporating CAC. Interaction and subgroup analyses were performed to explore whether the association between suPAR and outcomes differed according to the presence or absence of CAC and whether patients were on insulin therapy. Finally, we examined the incremental value of adding suPAR, CAC, or hs-CRP levels to clinical models for predicting all-cause mortality. C-statistics, continuous net reclassification improvement, and integrated discrimination improvement were calculated to evaluate the improvement in predictive ability of the models with and without biomarkers, using the R packages *survC1* and *survIDINRI*.<sup>29,30</sup>

Two-tailed  $P \leq 0.05$  was considered statistically significant. Analyses were performed using IBM SPSS Statistics Version 24 (Armonk, NY), SAS 9.4 (SAS Institute, Cary, NC), and R 3.2.2 (R Core Team, Vienna, Austria).

## Results

### The suPAR and Clinical Characteristics in African Americans With T2D

Table 1 displays characteristics of the 500 AA-DHS participants stratified by suPAR tertiles. Participants with higher suPAR levels were more likely to be female, smokers, have hypertension, history of CAD, lower eGFR, and elevated urine albumin-creatinine ratio. Although mean hemoglobin A<sub>1c</sub>, fasting glucose levels, and age at diabetes diagnosis were similar among groups, participants in the highest suPAR tertile were significantly more likely to be on insulin therapy (52% versus 31% for the lowest tertile). Notably, suPAR levels were higher in participants on insulin therapy regardless of glycemic control (Figure 1) and did not correlate with hemoglobin A<sub>1c</sub>, fasting glucose, or duration of diagnosis of T2D. In multivariable analyses suPAR levels were independently associated with female sex, current smoking, insulin use, lower eGFR, higher urine albumin-creatinine ratio and higher CAC (Table 2). The percentage of variance in suPAR levels explained by the multivariable model is 21.3%.

### SuPAR and Calcified Atherosclerotic Plaque

Participants with higher suPAR levels had higher median coronary artery, carotid artery, and infrarenal aorta calcium scores (Table 1). The correlation between suPAR and calcium plaque mass was modest, with correlation with the overall calcified plaque burden ( $r=0.20$ ,  $P<0.001$ ), aortic calcification ( $r=0.22$ ,  $P<0.001$ ), CAC ( $r=0.18$ ,  $P<0.001$ ), and carotid calcification ( $r=0.15$ ,  $P=0.001$ ). After adjusting for clinical characteristics including eGFR, urine albumin-creatinine ratio,

**Table 1.** Demographic and Clinical Characteristics of the AA-DHS Cohort, Stratified by suPAR Tertiles

Variable	Full Sample (n=500)	suPAR Tertiles			P Value*
		suPAR ≤2150 (n=168)	suPAR 2151 to 2866 (n=165)	suPAR >2866 (n=167)	
Age, y	56 (10)	54 (9)	56 (10)	57 (10)	0.012
Male, n (%)	220 (44%)	91 (54%)	69 (42%)	60 (36%)	0.003
Body mass index, kg/m <sup>2</sup>	36 (9)	34 (8)	36 (9)	36 (9)	0.085
Current smoker, n (%)	117 (23%)	34 (20%)	33 (20%)	50 (30%)	0.050
Hypertension, n (%)	421 (84%)	128 (76%)	146 (88%)	147 (88%)	0.002
History of coronary artery disease, n (%)	156 (31%)	48 (29%)	41 (25%)	67 (40%)	0.007
Prior stroke, n (%)	40 (8%)	9 (5%)	15 (9%)	16 (10%)	0.297
High-density lipoprotein, mg/dL	48 (14)	47 (14)	49 (13)	48 (15)	0.560
Low-density lipoprotein, mg/dL	107 (38)	105 (38)	108 (35)	109 (41)	0.557
Statin therapy, n (%)	254 (51%)	83 (49%)	84 (51%)	87 (52%)	0.885
Estimated glomerular filtration rate, mL/min per 1.73 m <sup>2</sup>	91 (22)	97 (19)	93 (21)	83 (24)	<0.001
Urine albumin-creatinine ratio, mg/g	145 (537)	46 (109)	115 (403)	275 (816)	<0.001
Hemoglobin A <sub>1c</sub> , %	8.1 (2.0)	7.9 (1.7)	8.2 (2.0)	8.3 (2.2)	0.147
Fasting glucose, mg/dL	151 (67)	147 (68)	150 (58)	156 (7)	0.417
Insulin treatment, n (%)	201 (40%)	52 (31%)	62 (38%)	87 (52%)	<0.001
Age at diabetes mellitus diagnosis, years	46 (10)	45 (9)	47 (10)	45 (10)	0.061
Coronary artery calcium score (CAC)	53 [2, 690]	19 [0, 255]	60 [3, 505]	157 [3, 1656]	<0.001
CAC of 0, n (%)	109 (22%)	51 (30%)	28 (17%)	30 (18%)	0.003 <sup>‡</sup>
CAC of 1 to 100, n (%)	170 (34%)	54 (32%)	65 (39%)	51 (31%)	
CAC of 101 to 300, n (%)	59 (12%)	22 (13%)	21 (13%)	16 (10%)	
CAC of >300, n (%)	162 (32%)	41 (24%)	51 (31%)	70 (42%)	
Aortic calcium score	997 [27, 6350]	347 [5, 2502]	1501 [18, 5817]	2391 [181, 10 857]	<0.001
Carotid artery calcium score	4 [0, 96]	0 [0, 43]	7 [0, 136]	10 [0, 174]	0.001
Principal component for aorta, carotid, and coronary calcium scores	-1.89 [-2.15, -0.49]	-2.07 [-2.16, -1.34]	-1.81 [-2.13, -0.29]	-1.62 [-2.12, 1.11]	<0.001
hs-CRP, mg/dL	0.5 [0.2, 1.09]	0.41 [0.18, 0.99]	0.49 [0.18, 1.27]	0.64 [0.25, 1.12]	0.089
suPAR, pg/mL	2506 [2014, 3195]	1821 [1604, 2015]	2506 [2322, 2657]	3495 [3194, 4118]	<0.001

Values are mean (SD), or n (%) as noted. Coronary artery calcium score (CAC), soluble urokinase plasminogen activator receptor (suPAR), and C-reactive protein (hs-CRP) are reported as median [25th, 75th percentile]. AA-DHS indicates African American-Diabetes Heart Study.

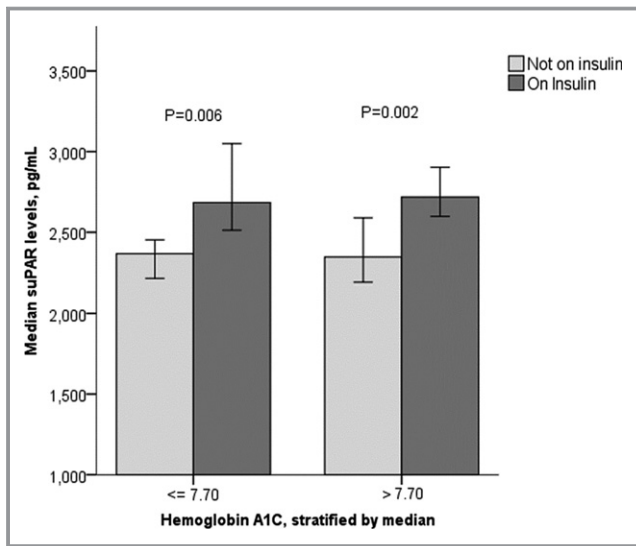
\*P-values are derived from ANOVA for normally distributed variables and the Mann-Whitney U test for nonnormally distributed variable.

<sup>‡</sup>P-value was derived from the Pearson chi-square test.

and hs-CRP, suPAR levels remained significantly associated with overall calcified plaque burden ( $\beta=0.010$ ,  $P<0.001$ ), CAC ( $\beta=0.004$ ,  $P=0.006$ ), aortic calcium plaque ( $\beta=0.001$ ,  $P<0.001$ ), but not carotid calcification ( $\beta=0.009$ ,  $P=0.087$ ). When examining the areas under the curve for predicting the presence of arterial calcification (calcium score >0), only age, eGFR, suPAR levels, and current smoking (in order of decreasing areas under the curve) were significantly associated (Table 3). Using the Youden index to identify optimal

cutoffs for suPAR yielded a suPAR level of 2595 pg/mL with a sensitivity of 69% and specificity of 61% for CAC >1000, and a sensitivity of 61% and specificity of 52% for CAC <100.

Finally, in the subset of 273 AA-DHS participants who returned for follow-up exams and a repeat computed tomography scan after  $\approx 5$  years, the median increase in total calcified plaque was 1832 [interquartile range 417-4930]. Enrollment suPAR levels were significantly correlated with the change in total calcified plaque ( $r=0.206$ ,  $P=0.001$ ). Adjusting



**Figure 1.** Median soluble urokinase-type plasminogen activator receptor (suPAR) levels stratified by hemoglobin A<sub>1c</sub> and insulin treatment. Error bars represent 95% confidence intervals.

for age, sex, body mass index, eGFR, smoking, and statin use, baseline suPAR levels remained significantly associated (odds ratio 1.82, 95% confidence interval (CI) [1.08-3.35]) with an increase in total calcified plaque score >1832 (median) at 5 years.

### SuPAR, Coronary Arterial Calcification, and Outcomes in T2D

After a median follow-up of 6.8 years (range 0.1-8.3), a total of 68 deaths were recorded: 13 with baseline suPAR ≤2150 pg/mL (first tertile), 20 in the second tertile (2150-2866 pg/mL), and 35 in the third tertile (>2866 pg/mL). In univariable analysis, participants in the higher tertiles of suPAR had a higher mortality rate (Figure 2). Similarly, when stratifying participants by CAC tertiles, increasing CAC was associated with shorter survival (Figure 2). After adjusting for demographics, clinical characteristics including kidney function, hs-CRP, and insulin use, suPAR levels (per 100% increase) remained significantly associated with a 2.65-fold increase in risk of all-cause mortality (Table 4). This association remained statistically significant even after adjusting for CAC, which was not an independent predictor of outcomes. Duration of T2D since diagnosis did not attenuate the association between suPAR and all-cause death. Of note, eGFR was no longer a significant predictor of death after adjusting for suPAR levels. In sensitivity analyses we found no significant interaction among suPAR, the presence of coronary calcified plaque, or treatment with insulin therapy in association with all-cause mortality.

The incremental value of adding suPAR to a model containing traditional risk factors and clinical characteristics in prediction of all-cause mortality was assessed. Addition of

**Table 2.** Characteristics Associated With suPAR Levels in African Americans With T2D

Variables	suPAR, Per 100% Increase	
	β, P Value	95% CI
Age, per 10 years	-0.023, 0.460	-0.086, 0.039
Male	-0.146, 0.005*	-0.246, -0.045*
Body mass index, per 5 kg/m <sup>2</sup>	0.012, 0.414	-0.017, 0.041
Hypertension	0.102, 0.123	-0.028, 0.231
Current smoking	0.211, <0.001*	0.099, 0.323*
History of coronary artery disease	-0.018, 0.743	-0.124, 0.089
Statin therapy	-0.123, 0.016*	-0.224, -0.023*
HDL, per mg/dL	0.001, 0.520	-0.002, 0.005
LDL, per mg/dL	0.000, 0.731	-0.002, 0.001
Hemoglobin A <sub>1c</sub> , per %	-0.003, 0.838	-0.030, 0.024
Insulin treatment	0.178, 0.001*	0.072, 0.285*
Duration since diagnosis of T2D, per 1 year	0.003, 0.358	-0.004, 0.010
Estimated glomerular filtration rate, per 10 mL/min per 1.73 m <sup>2</sup>	-0.068, <0.001*	-0.091, -0.045*
Urine albumin-creatinine ratio, per 10 mg/g	0.001, 0.024*	0.000, 0.002*
hs-CRP, per 100% increase (log <sub>2</sub> )	0.018, 0.603	-0.049, 0.085
CAC, per 100	0.004, 0.003*	0.001, 0.007*

The percentage of variance in suPAR (soluble urokinase plasminogen activator receptor) levels explained by the multivariable model is 21.3%. CAC indicates coronary artery calcium score; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T2D, type 2 diabetes mellitus. \*Statistically significant.

**Table 3.** Area Under the Curve for suPAR and Clinical Characteristics Predicting Presence of Arterial Calcification

Variable	Total Calcium Plaque Score >0	
	AUC, P Value	95% CI
Age	0.747, <0.001	0.667, 0.827
Estimated glomerular filtration rate	0.657, 0.003	0.575, 0.739
suPAR	0.621, 0.021	0.523, 0.719
Current smoking	0.611, 0.034	0.527, 0.696
hs-CRP	0.584, 0.110	0.491, 0.676
Male sex	0.567, 0.201	0.478, 0.655
Body mass index	0.561, 0.241	0.462, 0.661
Insulin therapy	0.559, 0.257	0.46, 0.658
Hypertension	0.530, 0.565	0.424, 0.636
Urine albumin-creatinine ratio	0.518, 0.736	0.421, 0.614
Hemoglobin A <sub>1c</sub>	0.466, 0.511	0.359, 0.573
LDL	0.462, 0.468	0.369, 0.555
Body mass index	0.460, 0.450	0.339, 0.582

CI indicates confidence interval; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; suPAR, soluble urokinase plasminogen activator receptor.

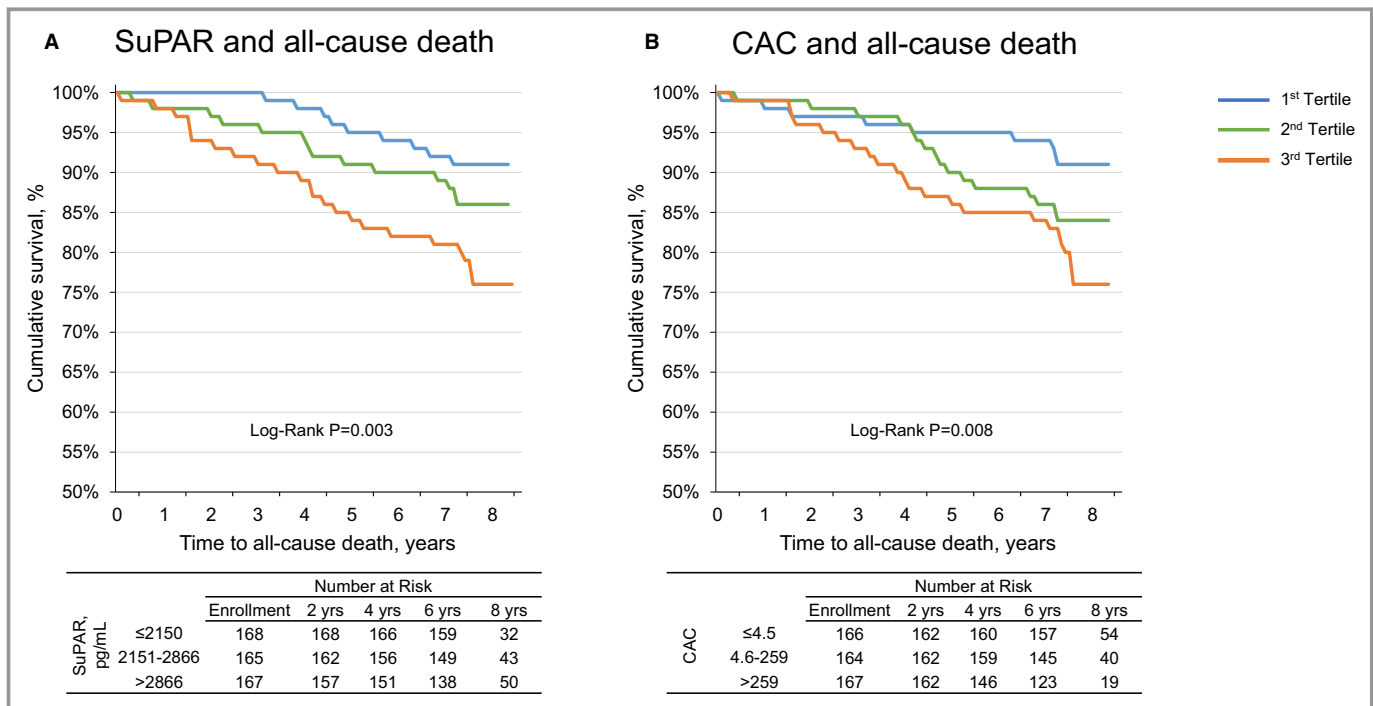
suPAR to a baseline clinical model significantly improved the C-statistic for all-cause death ( $\Delta 0.050$ , 95% CI [0.001, 0.100]), whereas addition of CAC ( $\Delta 0.005$ , 95% CI [-0.016, 0.027]) or hs-CRP ( $\Delta -0.001$ , 95% CI [-0.009, 0.007]) did not

(Table 5). The net reclassification improvement with addition of suPAR was significant (48%, 95% CI [33%, 56%]), whereas the net reclassification improvement with CAC was not (61%, 95% CI [-47%, 72%]). The integrated discrimination improvements for suPAR or CAC were not statistically significant.

### Discussion

In this prospective study of African Americans with T2D, we found suPAR to be a significant predictor of mortality, more strongly associated with all-causes of death than established biomarkers such as hs-CRP and CAC. SuPAR significantly improved risk stratification beyond traditional cardiovascular disease risk factors, and concentrations exhibited modest correlations with CAC. Elevated levels were associated with increasing arterial calcification after 5-year follow-up. Finally, we found insulin treatment to be a major determinant of suPAR levels in patients with T2D, independent from hemoglobin A<sub>1c</sub> or age at diagnosis.

This is the first study of which we are aware comparing suPAR with CAC for association with all-cause mortality. We and others have previously reported that suPAR levels are predictive of outcomes independent from traditional risk factors and add incremental benefit to models adjusted for biomarkers including hs-CRP, raising the C-statistic by 0.050.<sup>11-13,17,31</sup> Conversely, addition of hs-CRP to fully-adjusted models led to a change in C-statistic in the range



**Figure 2.** The suPAR concentration, coronary artery calcium score, and all-cause mortality. Kaplan-Meier survival curves for (A) soluble urokinase-type plasminogen activator receptor (suPAR), and (B) coronary artery calcium (CAC), stratified by tertiles.

**Table 4.** suPAR and All-Cause Death in African Americans With T2D

Variable	All-Cause Death	
	HR, P Value	95% CI
<b>Model 0: suPAR (unadjusted)</b>		
suPAR, per 100% increase (log <sub>2</sub> )	2.98, <0.001*	2.00, 4.43*
<b>Model 1: clinical characteristics+hs-CRP</b>		
Age, per 10 years	1.39, 0.066	0.98, 1.97
Male	2.16, 0.010*	1.20, 3.87*
Body mass index, per 5 kg/m <sup>2</sup>	1.03, 0.775	0.87, 1.21
Hypertension	1.21, 0.642	0.54, 2.71
Current smoking	2.05, 0.009*	1.20, 3.53*
History of coronary artery disease	1.57, 0.105	0.91, 2.69
Statin therapy	0.46, 0.007*	0.26, 0.81*
HDL, per mg/dL	1.01, 0.347	0.99, 1.03
LDL, per mg/dL	0.99, 0.005*	0.98, 1.00*
Hemoglobin A <sub>1c</sub> , per %	1.04, 0.654	0.88, 1.22
Insulin treatment	2.52, 0.002*	1.40, 4.51*
Duration since diagnosis of T2D, per 1 year	0.98, 0.230	0.94, 1.02
Estimated glomerular filtration rate, per 10 mL/min per 1.73 m <sup>2</sup>	0.91, 0.171	0.79, 1.04
Urine albumin-creatinine ratio, per 10 mg/g	1.00, 0.818	1.00, 1.01
hs-CRP, per 100% increase (log <sub>2</sub> )	0.98, 0.911	0.68, 1.42
<b>Model 2: clinical characteristics+hs-CRP+suPAR</b>		
suPAR, per 100% increase (log <sub>2</sub> )	2.65, <0.001*	1.63, 4.29*
<b>Model 3: clinical characteristics+hs-CRP+suPAR+CAC</b>		
suPAR, per 100% increase (log <sub>2</sub> )	2.65, <0.001*	1.62, 4.35*
CAC, per 100% increase (log <sub>2</sub> )	1.01, 0.77	0.94, 1.09
<b>Interaction and subgroup analyses</b>		
suPAR×CAC >0	1.10, 0.87	0.36, 3.37
suPAR×insulin treatment	0.92, 0.84	0.39, 2.18

Models 2 and 3, and the interaction analyses include the clinical characteristics in model 1 in addition to the listed variables. CAC indicates coronary artery calcium scores; CI, confidence interval; HDL, high-density lipoprotein; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; suPAR, soluble urokinase plasminogen activator receptor; T2D, type 2 diabetes mellitus.

\*Statistically significant at  $P < 0.05$ .

of 0.004.<sup>32</sup> In the present AA-DHS analysis, neither hs-CRP or CAC were independent predictors of all-cause mortality or improved risk discrimination after adjusting for known clinical characteristics such as smoking, insulin use, and kidney function, findings that contrast with previously reported associations.<sup>33,34</sup> This likely relates to the relatively small sample and low number of events in this subset of the AA-

DHS. Given these limitations, our findings do not contradict the well-established association between CAC and adverse outcomes but, rather, highlight the strong association between suPAR and all-cause mortality.

The pathophysiologic mechanisms underlying association between suPAR and adverse cardiovascular outcomes and mortality are unknown. Its membrane-bound form, uPAR, is present in multiple cell types, including immunologically active cells and endothelial cells, and encompasses physiologic roles impacting the coagulation cascade through the plasminogen activating pathway, cell signaling through integrins, and cell motility.<sup>35,36</sup> Although it may reflect immune activation, suPAR levels remain stable during episodes of sudden stress such as acute myocardial infarction or surgery; thus, suPAR differs from other inflammatory biomarkers such as hs-CRP and interleukin-6, which are acute-phase reactants.<sup>37,38</sup> SuPAR has been shown to scavenge vitronectin, a glycoprotein involved in coronary atherosclerosis.<sup>35</sup> Asymmetric dimethylarginine, a marker of oxidative stress, has also been shown to correlate with suPAR levels<sup>39</sup>; suPAR levels correlate with CAD risk factors and subclinical vascular dysfunction and can be modified by smoking cessation.<sup>13,40,41</sup> In this study baseline suPAR levels were associated with increased CAC at follow-up, consistent with a presumed role in atherosclerosis.<sup>35</sup> Several studies identified an association between suPAR and incident T2D.<sup>42,43</sup> In this study, individuals receiving insulin therapy had significantly higher suPAR concentrations, likely reflecting chronic inflammation and/or insulin resistance.

There is a consistent association between suPAR levels and adverse outcomes in the general population, critically ill patients, and those with CAD, HIV infection, malignancy, and chronic kidney disease; these effects are not dampened in multivariable analyses. Moreover, the multivariable models identifying determinants of suPAR levels that incorporate risk factors and clinical characteristics relevant to cardiovascular outcomes only explain a low percentage of the variance in suPAR levels. Thus, it appears likely that suPAR levels reflect cardiac and extracardiac pathophysiologic processes that are unaccounted for by known clinical characteristics, and therefore, may provide significant improvement to current risk stratification models.<sup>9-16,41,44,45</sup> While we await additional studies to elucidate the pathogenic role of suPAR or the pathways its levels represent, we envision the potential for use of suPAR concentrations in risk stratification to personalize care and optimize resource allocation.

## Strengths and Limitations

The strengths of this report include its community-based African American study sample, a cohort with access to healthcare based on high rates of treatment for hypertension, hyperlipidemia, and hyperglycemia. The AA-DHS is also among

**Table 5.** Risk Discrimination for All-Cause Mortality in African Americans With Type 2 Diabetes Mellitus

Model	All-Cause Mortality	
	C-Statistic (95% CI)	ΔC-Statistic (95% CI)
Model 1: risk factors only	0.689 (0.592, 0.784)	...
Model 2: RF and hs-CRP	0.678 (0.579, 0.780)	−0.001 (−0.009, 0.007)
Model 3: RF and CAC	0.694 (0.595, 0.793)	0.005 (−0.016, 0.027)
Model 4: RF and suPAR	0.739 (0.647, 0.831)	0.050 (0.004, 0.100)
Model 5: RF, suPAR, hs-CRP and CAC	0.740 (0.643, 0.838)	0.052 (0.002, 0.102)

Models 1 through 4 include age, sex, body mass index, smoking history, hypertension, baseline estimated glomerular filtration rate, low-density and high-density lipoprotein levels (risk factors [RF]). Models 2 to 4 include aforementioned risk factors in addition to hs-CRP (high-sensitivity C-reactive protein), CAC (coronary artery calcium score), and suPAR (soluble urokinase plasminogen activator receptor) levels, respectively. Model 5 includes RF, suPAR, hs-CRP, and CAC levels. The reported change in C-statistic is relative to the RF-only model. CI indicates confidence interval.

the most extensively phenotyped cohorts of individuals with T2D, including measures of albuminuria, hs-CRP, and CAC; repeat measures were available in a subset. Participants had generally preserved kidney function, reducing effects of chronic kidney disease on mortality. The major limitations are the relatively small sample size with low numbers of deaths and the inability to distinguish death from cardiovascular and noncardiovascular causes. These precluded performance of further subgroup and sensitivity analyses. In addition, information on structural heart disease such as ejection fraction was not available. Although this cohort is focused on the understudied African American population, associations between suPAR and outcomes, specifically both all-cause and cardiovascular mortality, have been described in multiple ethnic groups worldwide.<sup>9–17</sup> Nonetheless, the present findings warrant replication in a larger, multiethnic cohort before justifying risk stratification based on suPAR in the clinic.

## Conclusions and Clinical Implications

SuPAR levels are strong predictors of all-cause mortality in African Americans with T2D and add incremental value to risk stratification beyond CAC and hs-CRP. Whether measuring suPAR will alter management in patients with T2D warrants additional study.

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## Disclosures

Reiser is cofounder of TRISAQ, a biotechnology company that develops suPAR products. The remaining authors have no disclosures to report.

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