# Soluble Urokinase Plasminogen Activator Receptor Predicts Cardiovascular Events, Kidney Function Decline, and Mortality in Patients With Type 1 Diabetes

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## OBJECTIVE

**Soluble urokinase plasminogen activator receptor (suPAR) is an important inflammatory biomarker implicated in endothelial and podocyte dysfunction. However, suPAR's predictive qualities for complications in type 1 diabetes have yet to be determined. We investigated the prognostic value of suPAR for the development of cardiovascular events, decline in renal function, and mortality in patients with type 1 diabetes.**

## RESEARCH DESIGN AND METHODS

**We included 667 patients with type 1 diabetes with various degrees of albuminuria in a prospective study. End points were cardiovascular events (cardiovascular death, nonfatal acute myocardial infarction, nonfatal stroke, or coronary or peripheral arterial interventions), estimated glomerular filtration rate (eGFR) decline ≥30%, progression from lower to higher albuminuric state, development of end-stage renal disease (ESRD), and mortality. Follow-up was 5.2–6.2 years. Results were adjusted for known risk factors. Hazard ratios (HRs) are presented per doubling of suPAR with 95% CI. Relative integrated discrimination improvement (rIDI) was calculated.**

## RESULTS

**Quantification of suPAR was available in all participants; median (interquartile range) was 3.4 ng/mL (2.7–4.5). The adjusted HR (95% CI) for cardiovascular events ($n = 94$), progression in albuminuria ($n = 36$), eGFR decline ($n = 93$), ESRD ($n = 23$), and mortality ($n = 58$) were 3.13 (1.96–5.45, $P < 0.001$), 1.27 (0.51–3.19, $P = 0.61$), 2.93 (1.68–5.11, $P < 0.001$), 2.82 (0.73–11.9, $P = 0.13$), and 4.13 (1.96–8.69, $P < 0.001$), respectively. rIDI was significant for cardiovascular events (22.6%, $P < 0.001$), eGFR decline (14.4%, $P < 0.001$), and mortality (23.9%, $P < 0.001$).**

## CONCLUSIONS

**In patients with type 1 diabetes and a broad range of albuminuria, a higher level of suPAR is a significant and independent risk factor for cardiovascular events, decline in eGFR ≥30%, and mortality. In addition, suPAR contributes significantly to discrimination for the end points.**


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Type 1 diabetes is a serious and well-known risk factor for development of cardiovascular disease (1,2) and diabetic kidney disease (DKD) (3) and a strong risk factor for mortality (4). Furthermore, as type 1 diabetes often is diagnosed at a young age (5), patients are at high risk of developing complications early, markedly raising the mortality rate (6). This underscores the need for earlier and improved risk stratification of these patients in order to enable precipitated and targeted treatment for prevention of complications and death.

It has previously been shown that individuals with type 1 diabetes generally exhibit a high state of inflammation and oxidative stress (7), in part due to the autoimmunological nature of the disease (7), which in turn has been associated with a sizable component in the development of micro- and macroangiopathy (8). Therefore, it is of interest to investigate and target inflammatory biomarkers and their pathways in hopes of better prediction and treatment of type 1 diabetes.

One of the proposed entry points is soluble urokinase plasminogen activator receptor (suPAR), an emerging biomarker associated with a myriad of inflammatory pathways (9). It originates from urokinase plasminogen activator receptor (uPAR), a membrane receptor expressed mainly on immune (10) and endothelial cells (11), which during inflammation is released in circulation in its soluble form, suPAR (12). Current methods of determining inflammatory status commonly use C-reactive protein (CRP), which is widely applied in several clinical disciplines to assess inflammation and severity of disease (13). However, the stability and unspecificity of suPAR may allow for a broader assessment of the inflammatory state, as it has been correlated with the development of several pathological conditions, e.g., cardiovascular disease (14,15), mortality (16), type 2 diabetes (16,17), DKD (18), cancer (19), and sepsis (20), in addition to being a strong marker of mortality and admission time in acutely admitted medical patients (20,21). As such it has been shown to be an attractive biomarker for use in both general risk stratification settings and for treatment optimization of patients, in chronic outpatient settings as well as acute settings.

Furthermore, high suPAR levels have been linked to the development of chronic kidney disease (CKD), suggested by its activation of podocytes in pathological conditions (22). Podocytes have additionally been theorized to be a main mechanism and influence in the development of DKD specifically (23), possibly driven by, or associated with, high suPAR levels (24). As such it is suggested that suPAR has a role in the early prediction of kidney function decline (24), as indicated by its ability to predict development of microalbuminuria in normoalbuminuric individuals with type 2 diabetes (18).

However, suPAR is not without its controversies. Several studies have correlated higher suPAR levels with decreased kidney function, and another has shown that estimated glomerular filtration rate (eGFR) does not affect suPAR in urine or circulation (25–28). Although none of the studies show a causal association between kidney function and suPAR, it can be measured in urine, which is cause for prudence when evaluating the biomarker in individuals with impaired kidney function.

Aiming further attention at suPAR's prognostic aptitude, Eapen et al. (14) have shown that individuals with a suPAR level $\geq$3.5 ng/mL at baseline had a hazard ratio (HR) of 3.2 for the development of myocardial infarction, and an HR of 2.6 for cardiac mortality, compared with individuals with a level $<$3.5. Another study found the risk of new-onset diabetes in a nonsmoking population of middle-aged individuals to be 3.5 times higher in participants in the highest quartile of suPAR compared with the lowest, after comprehensive adjustment (17).

The existing knowledge indicates suPAR as being a potent risk factor in various conditions, indicating possible clinical applications for risk stratification in certain patient groups. However, the clinical value has not been robustly resolved in several populations, including patients with type 1 diabetes. Therefore, in this study, we analyze the predictive qualities of suPAR in relation to the development of cardiovascular events, development and progression of renal impairment, and mortality in patients with type 1 diabetes.

## RESEARCH DESIGN AND METHODS

### Participants

From 2009 to 2011, we recruited 667 patients with type 1 diabetes from the outpatient clinic at Steno Diabetes Center Copenhagen. The details of the study have previously been described (29). In short, participants had type 1 diabetes according to World Health Organization criteria and were $\geq$18 years of age. The cohort was stratified by levels of albuminuria (normo-, micro-, and macroalbuminuria). Patients with end-stage renal disease (ESRD), defined as receiving dialysis or renal transplantation or GFR $<$15 mL/min/1.73 $m^2$, were excluded. The study complied with the Declaration of Helsinki, and all activities, as well as the research protocol, were approved by the local ethics committee. All patients gave informed written consent.

### Analyses at Baseline

suPAR was measured using Conformité Européenne and in vitro diagnostic–approved suPARnostic ELISA kits (ViroGates, Birkerød, Denmark) according to the manufacturer's protocol. Quantification of suPAR was available for all 667 patients. LDL cholesterol, serum creatinine, and $HbA_{1c}$ were specified from venous blood samples using standardized methods. Urinary albumin excretion rate (UAER) was determined in three consecutive 24-h urine collections and analyzed with enzyme immunoassay (Vitros, Raritan, NJ). eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation based on serum creatinine. CRP levels were measured using a particle-enhanced immunoturbidimetry hs-CRP assay (Roche/Hitachi, Group Communications, Basel, Switzerland). Levels of suPAR and CRP were measured after one thawing cycle, and samples were stored at −80°C until analysis.

Participants were categorized as normoalbuminuric if UAER was $<$30 mg/24 h, as microalbuminuric if UAER was or previously had been recorded between 30 and 299 mg/24 h, and as macroalbuminuric if UAER was or previously had been recorded $\geq$300 mg/24 h in two out of three consecutive measurements. All patients classified as normoalbuminuric did not have any history of micro- or macroalbuminuria prior to enrollment in the study.

### Follow-Up

In 2016, patients were traced through the Danish National Death Register and the Danish National Health Register (30,31), from which data regarding mortality,

including date and cause of death, hospital admission, and ICD-10 diagnoses were obtained. Both registers have very high coverage and validity as all deaths and all hospital admissions in Denmark are captured. Deaths were classified as cardiovascular unless any other cause was determined. Cause of death was indefinitive in only three participants. The cardiovascular end point was defined as cardiovascular death, nonfatal acute myocardial infarction (ICD-10 I21–I24), nonfatal stroke (ICD-10 I61–I66), coronary interventions (procedural codes KFNA–D), or peripheral arterial interventions including amputations. ESRD was defined as CKD stage 5 (ICD-10 N18.5), chronic dialysis (procedural code BJFD2), kidney transplantation (procedural code KKAS 00, 10, and 20), or eGFR $<$15 mL/min/1.73 m$^2$.

Information regarding eGFR and urine albumin-to-creatinine ratio during follow-up was obtained at outpatient visits and was traced through electronic laboratory records. The remaining renal end points were defined as *1*) a decline in eGFR $\geq$30%, as proposed by Coresh et al. (32), and *2*) progression in albuminuria status, defined as progression from normo- to microalbuminuria or micro- to macroalbuminuria in two out of three consecutive measurements, or based on a single measurement of albuminuria at baseline compared with the first single measurement at a higher albuminuria status during follow-up. Unless otherwise stated, results for the progression in albuminuria end point throughout the paper are reported based on the former method. There were no cases of progression from normo- to macroalbuminuria recorded.

In the event of multiple end points being registered, only the first was included for analysis. Median (interquartile range [IQR]) follow-up was 5.1 years (4.7–5.6) for cardiovascular events, 5.3 years (2.7–6.2) for decline in eGFR $\geq$30%, 5.3 years (4.8–5.7) for ERSD, 5.8 years (2.5–6.4) for progression in albuminuria status, and 6.2 years (5.8–6.7) for mortality.

### Statistical Analysis

suPAR was presented as median with IQR and log2 transformed in later analyses to achieve normal distribution. Normally distributed variables are given as mean $\pm$ SD and categorical variables as total numbers with corresponding percentages. Baseline clinical characteristics were compared across quartiles of suPAR using ANCOVA and $\chi^2$ test for continuous and categorical variables, respectively.

HRs and 95% CI were calculated using Cox proportional hazards model for all end points and presented per doubling of suPAR. Adjustment included traditional risk factors and confounders: sex, age, diabetes duration, plasma LDL cholesterol, $HbA_{1c}$, systolic blood pressure, BMI, smoking status, UAER, eGFR, treatment with renin-angiotensin-aldosterone system inhibitiors (RAASi), and CRP.

Next, to calculate added prognostic impact of the biomarker, we used receiver operating characteristic (ROC) curves, applying C-statistics for area under the curve (AUC) analysis. Furthermore, the relative integrated discrimination improvement (rIDI) was calculated, having previously been suggested as a strong method for assessing new biomarkers in supplement to traditional risk factors (33). Finally, Kaplan-Meier functions and the log-rank test were applied to compare risks across quartiles of suPAR. We tested heterogeneity in the HRs for the influence of sex by introducing the appropriate interaction term in the Cox model.

A two-tailed $\alpha$-level of $\leq$0.05 was considered significant. Statistical analysis was performed using SAS software (version 9.4; SAS Institute, Cary, NC).

**Table 1—Baseline characteristics and follow-up information divided according to quartiles of suPAR**

| | Quartiles of suPAR | | | | |
|---|---|---|---|---|---|
| | $<$2.75 ng/mL | $\geq$2.75 to $<$3.5 ng/mL | $\geq$3.5 to $\leq$4.6 ng/mL | $>$4.6 ng/mL | *P* |
| Number of participants | 167 | 164 | 166 | 170 | |
| Female | 38 | 44 | 48 | 48 | 0.22 |
| Age (years) | 46 $\pm$ 13 | 55 $\pm$ 11 | 60 $\pm$ 11 | 59 $\pm$ 11 | $<$0.001 |
| Diabetes duration (years) | 20 $\pm$ 15 | 32 $\pm$ 14 | 38 $\pm$ 13 | 41 $\pm$ 12 | $<$0.001 |
| eGFR (mL/min/1.73 m$^2$) | 100 $\pm$ 15 | 92 $\pm$ 15 | 80 $\pm$ 19 | 56 $\pm$ 26 | $<$0.001 |
| UAER (mg/24 h) | 11 (7–20) | 13 (7–40) | 16 (9–74) | 64 (16–339) | $<$0.001 |
| $HbA_{1c}$ (mmol/mol) | 62 $\pm$ 13 | 65 $\pm$ 13 | 66 $\pm$ 12 | 65 $\pm$ 13 | 0.012 |
| $HbA_{1c}$ (%) | 7.8 $\pm$ 1.2 | 8.1 $\pm$ 1.2 | 8.2 $\pm$ 1.1 | 8.1 $\pm$ 1.2 | 0.012 |
| LDL cholesterol (mmol/L) | 2.5 $\pm$ 0.7 | 2.5 $\pm$ 0.8 | 2.4 $\pm$ 0.6 | 2.4 $\pm$ 0.9 | 0.21 |
| BMI (kg/m$^2$) | 25 $\pm$ 3 | 26 $\pm$ 4 | 26 $\pm$ 9 | 25 $\pm$ 25 | 0.51 |
| Antihypertensive drugs | 44 | 71 | 77 | 96 | $<$0.001 |
| RAASi | 41 | 66 | 72 | 89 | $<$0.001 |
| Smoking | 11 | 16 | 28 | 26 | $<$0.001 |
| Systolic blood pressure (mmHg) | 126 $\pm$ 15 | 130 $\pm$ 14 | 136 $\pm$ 19 | 135 $\pm$ 19 | $<$0.001 |
| Diastolic blood pressure (mmHg) | 75 $\pm$ 9 | 75 $\pm$ 8 | 74 $\pm$ 10 | 73 $\pm$ 10 | 0.014 |
| Follow-up | | | | | |
| Cardiovascular events, *n* (%) | 2 (1.2) | 12 (7.3) | 32 (19.3) | 48 (28.1) | $<$0.001 |
| Decline in eGFR $\geq$30%, *n* (%) | 6 (3.6) | 11 (6.7) | 19 (11.5) | 57 (33.3) | $<$0.001 |
| Progression in albuminuria, *n* (%) | 5 (3.1) | 7 (4.3) | 9 (5.4) | 15 (8.8) | 0.11 |
| ESRD, *n* (%) | 0 (0) | 0 (0) | 1 (0.6) | 37 (22.0) | $<$0.001 |
| Total mortality, *n* (%) | 2 (1.2) | 7 (4.3) | 12 (7.2) | 37 (21.6) | $<$0.001 |

Data represent percentage, mean $\pm$ SD, or median (IQR) unless otherwise indicated. *P* values are for trend across quartiles.

## RESULTS

### Baseline Characteristics

To illustrate baseline characteristics across the suPAR range, we divided the 667 patients into quartiles of suPAR (<2.75, ≥2.75 to <3.50, ≥3.50 to ≤4.60, and >4.60 ng/mL) in Table 1. Patients in higher quartiles were generally older (Q1 46 years, Q4 59 years; $P < 0.001$), had longer diabetes duration (Q1 20.2 years, Q4 40.5 years; $P < 0.0001$), had lower eGFR (Q1 99.6 mL/min/1.73 $m^2$, Q4 55.7 mL/min/1.73 $m^2$; $P < 0.0001$), and had higher $HbA_{1c}$ (Q1 62 mmol/mol, Q4 65 mmol/mol; $P = 0.012$), and a higher proportion were treated with RAASi (Q1 41%, Q4 89%; $P < 0.0001$). Furthermore, patients in higher quartiles had higher systolic blood pressure (Q1 126 mmHg, Q4 135 mmHg; $P < 0.001$) but lower diastolic blood pressure (Q1 75 mmHg, Q4 73 mmHg; $P = 0.014$). The sex distribution, LDL cholesterol, and BMI were comparable across quartiles.

### Follow-up Analysis

The comparison of the proportion of end points across quartiles of suPAR showed a large variance. Q4 consistently encompassed the highest number of events for all end points compared with the lower quartiles. The event rates across quartiles (Table 1) for cardiovascular events (2, 12, 32, and 48; $P < 0.001$), eGFR decline (6, 11, 19, and 47; $P < 0.001$), and mortality (2, 7, 12, and 37; $P < 0.001$) all increased linearly. This was also the case for progression in albuminuria (5,7,9,15); however, it was not significant ($P = 0.11$). Not presented in Table 1 are results for progression in albuminuria based on single measurements; the event rates over quartiles were comparable (23, 24, 27, and 28; $P = 0.90$), with a total of 102 events. For ESRD, all events but one were located in Q4 (0, 0, 1, and 37; $P < 0.001$). Kaplan-Meier plots (Fig. 1) illustrate end points across quartiles, with all end points apart from progression in albuminuria ($P = 0.11$) demonstrating highly significant trends evaluated with the log-rank test ($P < 0.001$).

### Risk Prediction and Prognostic Value

Cox regression analyses for all end points are presented in Table 2. Results for each of the end points are shown in unadjusted and stepwise adjusted models as indicated. Higher suPAR levels at baseline were associated with a highly significant risk prediction of cardiovascular events, eGFR decline, and mortality, with HR (95% CI) per doubling of suPAR 3.13 (1.96–5.45, $P < 0.001$), 2.93 (1.68–5.11, $P < 0.001$), and 4.13 (1.96–8.69, $P < 0.001$), respectively, in adjusted models also including CRP. HR (95% CI) for ESRD in the unadjusted model was significant (15.5 [7.93–30.4], $P < 0.001$); however, results were radically changed and significance was lost after adjustment (2.82 [0.73–11.9], $P = 0.13$). Furthermore, there was an apparent risk prediction for progression in albuminuria in the unadjusted model (HR 2.16 [1.28–3.65], $P = 0.004$); however, significance was lost after adjustment (HR 1.27 [0.51–3.19], $P = 0.61$).

Other significant risk factors for cardiovascular events were higher age, LDL cholesterol, $HbA_{1c}$, diabetes duration, and male sex ($P \leq 0.045$).

The rIDI analysis showed significant results for cardiovascular events, eGFR

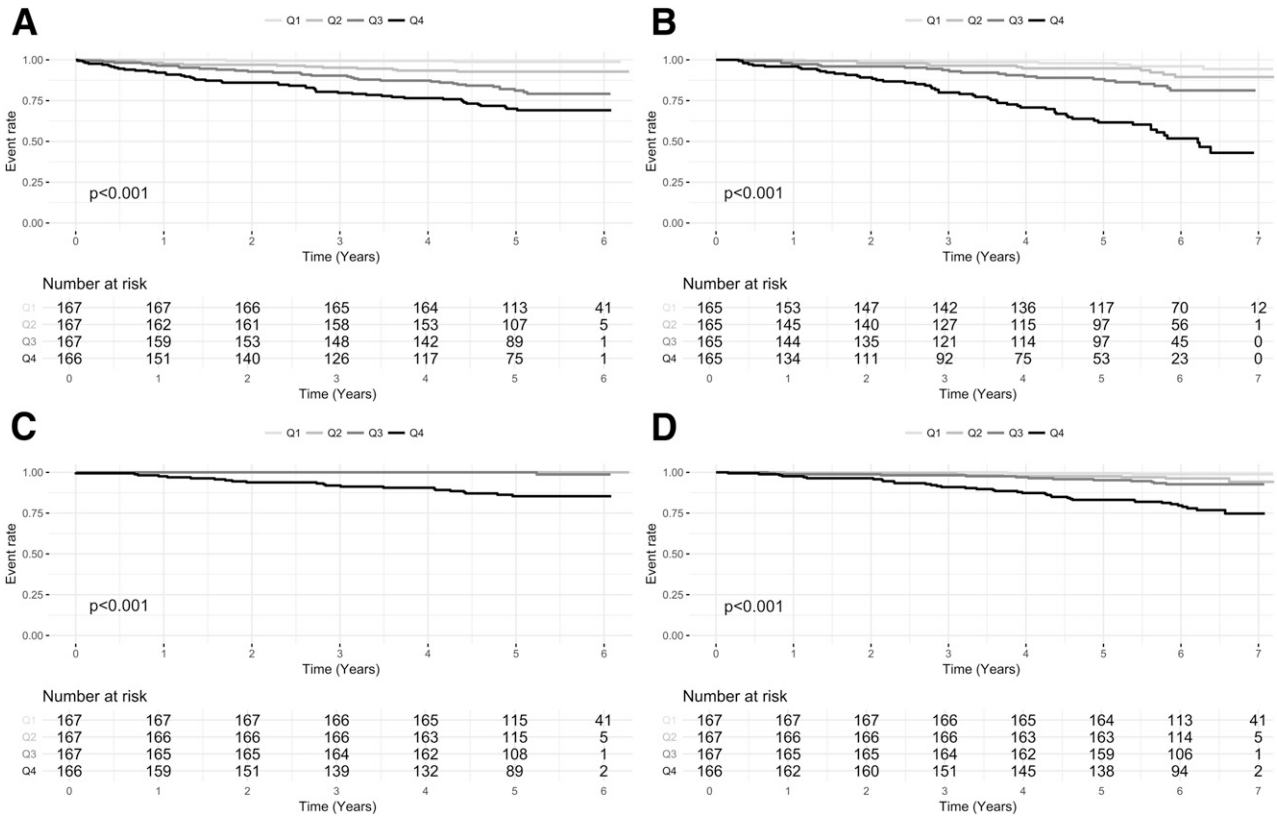


**Figure 1**—Kaplan–Meier failure function estimates for quartiles (Q) of suPAR for cardiovascular events (*A*), eGFR decline (*B*), ESRD (*C*), and mortality (*D*). *P* values are calculated by log-rank test across quartiles.

**Table 2—HR of suPAR to base-adjusted model, which included sex, age, diabetes duration, LDL, $HbA_{1c}$, systolic blood pressure, BMI, smoking, UAER, and prescribed RAASi**

| Model | Cardiovascular events | *P* | Progression in albuminuria | *P* | Decline in eGFR ≥30% | *P* | ESRD | *P* | Total mortality | *P* |
|---|---|---|---|---|---|---|---|---|---|---|
| *n* (%) | 94 (14.2) | | 36 (5.5) | | 93 (14.0) | | 23 (3.5) | | 58 (8.7) | |
| Unadjusted | 3.43 (2.52–4.66) | <0.001 | 2.16 (1.28–3.65) | 0.004 | 4.29 (3.14–5.84) | <0.001 | 15.5 (7.93–30.4) | <0.001 | 4.37 (2.95–6.46) | <0.001 |
| Adjusted | 3.15 (2.05–4.85) | <0.001 | 1.20 (0.55–2.61) | 0.65 | 3.75 (2.46–5.73) | <0.001 | 24.4 (8.31–71.8) | <0.001 | 3.57 (1.99–6.41) | <0.001 |
| Adjusted + eGFR | 3.03 (1.79–5.12) | <0.001 | 1.28 (0.51–3.23) | 0.61 | 2.80 (1.61–4.89) | <0.001 | 2.92 (0.77–11.0) | 0.09 | 3.89 (1.87–8.09) | <0.001 |
| Adjusted + eGFR + CRP | 3.13 (1.96–5.45) | <0.001 | 1.27 (0.51–3.19) | 0.61 | 2.93 (1.68–5.11) | <0.001 | 2.82 (0.73–11.9) | 0.13 | 4.13 (1.96–8.69) | <0.001 |
| rIDI (%) | 22.6 | <0.001 | 0.68 | 0.75 | 14.4 | <0.001 | 5.7 | 0.27 | 23.9 | <0.001 |

Further stepwise adjustment added eGFR and CRP respectively. HRs were calculated per doubling of suPAR and are presented with 95% CI. rIDI analysis of suPAR, in addition to a base model including sex, age, diabetes duration, LDL, $HbA_{1c}$, systolic blood pressure, BMI, smoking, UAER, prescribed RAASi, eGFR, and CRP.

decline, and mortality. The added discrimination slope contribution for these end points was 22.6% ($P < 0.001$), 14.4% ($P < 0.001$), and 23.9% ($P < 0.001$), respectively. In the case of progression in albuminuria and ESRD, low and insignificant discrimination contributions were demonstrated with 0.68% ($P = 0.75$) and 5.7% ($P = 0.27$), respectively.

The ROC and AUC analysis, presented in Fig. 2, showed significance for cardiovascular events, exhibiting AUC of 0.81 for the model with suPAR and 0.78 for the model without ($P = 0.017$). Results for the other end points were not significant ($P \geq 0.18$).

### Sensitivity Analysis

None of the tests for heterogeneity in relation to the influence of sex reached significance, not in unadjusted or adjusted analyses ($0.15 < P < 0.65$).

## CONCLUSIONS

The current study investigates the role of suPAR for prediction of complications in type 1 diabetes. We demonstrated that a higher suPAR level is a significant and independent risk marker for development of cardiovascular events, decline in eGFR, and mortality. Likewise, suPAR is a contributing factor in risk stratification models on top of established risk factors for the same end points evaluated with rIDI statistics. These results stand well in relation to existing literature, where suPAR has been correlated with the development of various conditions in populations without diabetes. In a cohort of 3,367 patients undergoing cardiac catherization, a suPAR level of ≥3.5 ng/mL was a significant predictor (HR 1.9) for the development of a composite end point including myocardial infarction or death after a mean follow-up of 2.1 years (14). In another publication, from the same cohort, it was shown that a higher suPAR level was also significantly associated with a larger annual decline in eGFR as well as an increased risk of developing CKD, defined as eGFR <60 mL/min/1.73 $m^2$ (34).

To the best of our knowledge, the predictive qualities of suPAR have never been evaluated in relation to the prospective development of diabetic complications in patients with type 1 diabetes. This is important considering the high risk of disease they exhibit compared with the general population (4). We have previously shown in a cross-sectional study of the same cohort that a higher level of suPAR was associated with the presence of cardiovascular disease, albuminuria, autonomic impairment, arterial stiffness, and myocardial impairment (35,36). In the current study, we traced longitudinal changes in kidney function and the development of hard cardiovascular end points and mortality, enabling us to demonstrate the predictive value of suPAR on top of existing risk factors.

Baseline data showed significant differences across quartiles of suPAR for age, diabetes duration, eGFR, UAER, smoking status, and systolic blood pressure, with a more favorable risk profile in patients in the lowest quartiles. However, a higher suPAR level was a consistent predictor even after comprehensive adjustment, emphasizing the independent value of suPAR for risk prediction.

Furthermore, AUC analysis of ROC curves showed increases for all end points and a significant increase for cardiovascular events, despite the already potent baseline model. ESRD showed significant correlation with higher event rate in the highest quartile of suPAR, whereas progression in albuminuria status was nonsignificant. In addition, although ESRD was not significant in our continuous analyses, all but one of the events developed in the group with the highest quartile of suPAR (>4.6 ng/mL). This relationship is clearly illustrated in the Kaplan-Meier analyses pictured in Fig. 1 and, in addition to our continuous Cox regression analyses, illustrates the usefulness and versatility of suPAR as a biomarker.

The results for the renal end points illustrate an interesting picture. We show significance for the prediction of eGFR decline, and for ESRD, we find a higher event rate associated with higher levels of suPAR, but analyses for the progression of albuminuria were insignificant after adjustment. It has previously been shown that elevated levels of suPAR predict the development of microalbuminuria in individuals at risk for developing type 2 diabetes (18). However, interestingly, Hayek et al. (34) highlight that patients with existing CKD at baseline (eGFR <60 mL/min/1.73 $m^2$) did not experience suPAR-related reduction in renal function compared with patients without CKD (eGFR ≥60 mL/min/1.73 $m^2$).

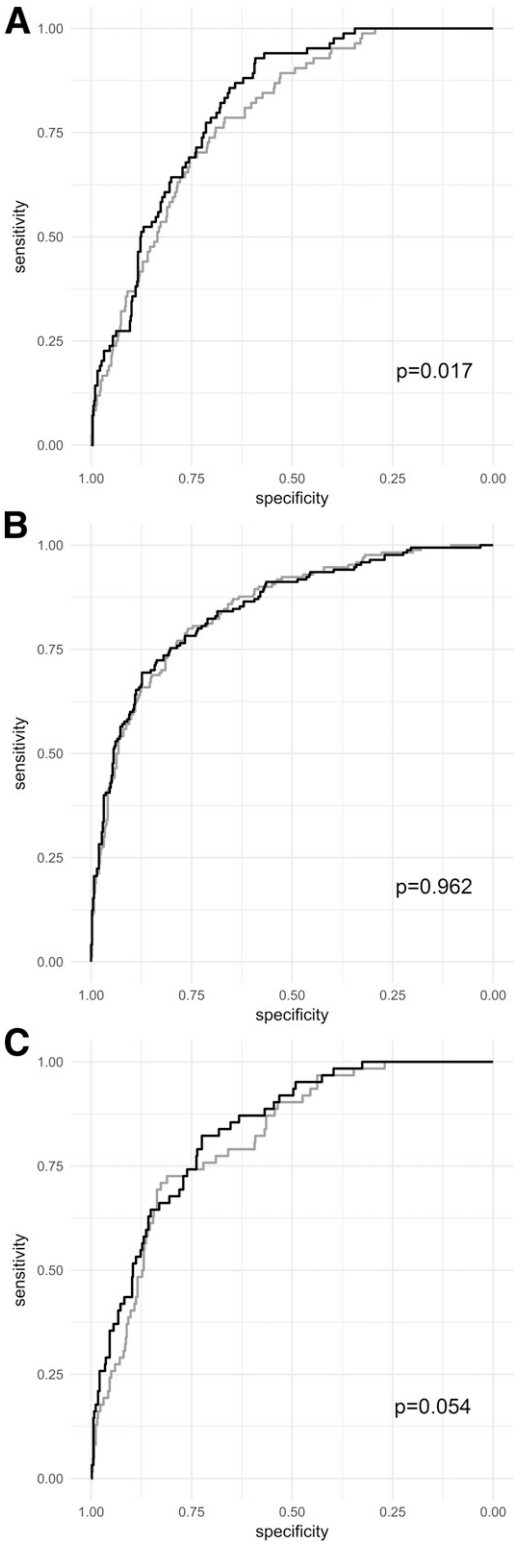


**Figure 2**—ROCs for base model (gray line) vs. base model including suPAR (black line). Base model includes sex, age, diabetes duration, LDL cholesterol, $HbA_{1c}$, systolic blood pressure, BMI, smoking, UAER, eGFR, treatment with RAASi, and CRP. *A*: ROC for cardiovascular events. *B*: ROC for eGFR decline ≥30%. *C*: ROC for mortality. *P* values were calculated comparing AUC for the respective models.

Hence, it is possible that suPAR is a more potent biomarker for early development of kidney disease, whereas the progression of albuminuria is a later complication and thus not associated with suPAR in the same extent.

Also to be noted is the threefold discrepancy of results between our progression in albuminuria end points described in the RESULTS. When progression was based on two out of three consecutive measurements, we demonstrated a clear, although insignificant, trend toward higher event rates in higher quartiles of suPAR, as compared with basing it on single high and low measurements, which results in numerous additional events spread evenly over the quartiles. It can be argued that there is a clear confounding factor in basing the end point on two out of three consecutive measurements, as those UAER measurements can be separated by up to 1 year. However, the same can be said, arguably to a greater extent, for the alternative, as albumin excretion in patients with diabetes is highly variable (3), and as such, single measurements are wholly unsuitable in this regard.

The current study indicates that suPAR might play an important role in risk stratifying individuals with type 1 diabetes. However, it is unclear what processes determine this relationship, especially in relation to kidney function. It has been theorized that when subjected to higher suPAR levels, $\alpha v\beta 3$ integrins present on kidney podocytes induce structural and pathological changes (22). Animal models have furthermore shown that targeting suPAR with antibodies leads to reduced proteinuria (22).

The mechanism relating suPAR to risk of cardiovascular morbidity and mortality has not been clarified, and the exact physiological role and physiology of uPAR and suPAR is not fully understood. In healthy individuals, uPAR is not generally expressed, although this radically changes after occurrence of inflammation or tissue injury. Different modes of action have been theorized, where one of the more intriguing is the relationship to vitronectin (37), a glycoprotein associated with, among others, coronary atherosclerosis. Vitronectin regulates and binds to uPAR, and it has been suggested that this binding is enhanced after uPAR has been bound by urokinase-like plasminogen activator (uPA) as well (38). Furthermore, suPAR has been linked to the formation of atherosclerotic lesions, where uPAR overexpression in the endothelial layers was demonstrated to contribute (39). These findings might

explain that the end point best predicted by suPAR was the cardiovascular outcome.

Despite these associations, it is unknown if there is a causal relationship between elevated suPAR levels and higher risk of disease, or if it merely is a marker of disease progression and is nonmodifiable (38). In particular, previous studies have demonstrated a strong association between decreased kidney function and elevated suPAR levels (25–27). suPAR is filtered through the kidney and can be measured in urine, although the precise mechanisms regarding its reabsorption in the tubuli, or lack thereof, remain to be clarified. Albeit an inverse correlation between kidney function and suPAR levels is demonstrated in our study, the results for prediction of outcome remain significant after adjustment for baseline eGFR, indicating that suPAR is not simply a filtration marker.

Smoking is a known risk factor for cardiovascular disease and was more common in the higher quartiles of suPAR. However, interestingly, it was not an independent risk factor for cardiovascular disease in our population.

Our study does not have the characteristics to clarify the pathophysiological effects of suPAR, but we identify suPAR as a potentially auspicious key player in the development of diabetic complications. Although requiring further research governing the specific mechanisms through which suPAR operates, the potential value of suPAR for risk stratification in patients with type 1 diabetes should not be diminished. Furthermore, measurements are performed using standard venipuncture, and standardized assays are commercially available, making it an accessible method to use in a clinical setting.

Strengths of this study include the large and well-defined cohort representing a broad segment of the population with type 1 diabetes exhibiting all stages of albuminuria. Moreover, no people were lost to follow-up. Limitations include the lack of generalizability due to recruitment from a single center, as well as suPAR concentrations being measured in stored plasma samples. However, suPAR is stable in frozen samples, even after long (years) storage duration and repeated cycles of freezing and thawing (40).

In summary, in a population of 667 patients with type 1 diabetes and a broad range of albuminuria, a higher level of suPAR is a highly significant and independent risk factor for cardiovascular events, mortality, and decline in kidney function evaluated as decline in eGFR $\geq$30%. In addition, it contributes marked, significant discrimination beyond traditional risk factors for the above-mentioned end points.

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**Duality of Interest.** J.E.-O. is a CSO, cofounder, and shareholder in ViroGates and is named inventor on patents on suPAR, owned by Copenhagen University Hospital Hvidovre. Outside of this study, P.R. reports having given lectures for AstraZeneca, Novo Nordisk, Eli Lilly, Bayer, and Boehringer Ingelheim; has served as a consultant for AbbVie, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Boehringer Ingelheim, Astellas, Janssen, and Novo Nordisk (all fees given to Steno Diabetes Center Copenhagen); and has equity interest in Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

**Author Contributions.** V.R.C., S.T., S.A.W., N.T., J.E.-O., F.P., T.W.H, J.J., and P.R. conceived and designed the research. V.R.C., S.T., S.A.W., N.T., F.P., T.W.H., and P.R. analyzed and interpreted the data. T.W.H. performed the statistical analysis. P.R. obtained funding and supervised the study. V.R.C. wrote the manuscript. All authors critically revised the manuscript for key intellectual content and approved the final version of the manuscript. V.R.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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