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Serum amyloid a and risk of death and end-stage renal disease in diabetic kidney disease

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Abstract

Aims—To determine if serum levels of serum amyloid A (SAA) predict death and end-stage renal disease in a cohort of people with diabetic kidney disease.

Methods—In a longitudinal cohort study of 135 participants with type 2 diabetes and diabetic kidney disease, serum samples were assayed for SAA. Censored time-to-event analyses in Cox-proportional hazard models were utilized to assess SAA as a predictor of the primary outcome of death and end-stage renal disease.

Results—Participants were 73% Mexican-American (99/135) and 55% men (75/135), with a mean ± SD age of 57 ± 7.5 years. At baseline, participants had hemoglobin A1c of 8.6 ± 2.3%, systolic blood pressure of 153 ± 27 mm Hg, body mass index of 31 ± 9 kg/m^2, median urine-albumin-to-creatinine ratio of 1861 mg/g (interquartile range 720–3912 mg/g), and estimated glomerular filtration rate of 55.7 ± 22.3 ml/min/1.73 m^2. Over a median duration of follow-up of 3.5 years, 44% (60/135)of participants experienced a primary outcome event. The hazards ratio for the primary outcome was 3.03 (95% CI 1.43–6.40, p = 0.003) in the highest (>1.0 μg/ml) compared to the lowest (<0.55 μg/ml) SAA tertile in a model adjusted for urine-albumin-to-creatinine ratio, estimated glomerular filtration rate, age, sex, and race/ethnicity. Addition of SAA as a covariate improved the model *C-statistic* (Δ c = 0.017).

Conclusions—In a longitudinal cohort study of participants with type 2 diabetes and DKD, higher levels of serum SAA predicted higher risk of death and ESRD. SAA is a promising targetable biomarker for DKD.

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Keywords

Biomarkers; Chronic kidney disease; Inflammation; Risk-stratification; Diabetes

1. Introduction

The prevalence of diabetic kidney disease (DKD) has become progressively higher over the past two decades despite substantive efforts to improve diabetes care (de Boer et al., 2011; National Kidney F, 2012). In the United States, 44% of end-stage renal disease (ESRD) cases are presently attributed to diabetes (Collins, Foley, Gilbertson, & Chen, 2015; de Boer et al., 2011). Risks of all-cause mortality and cardiovascular disease are also strongly related to DKD (Foley, Parfrey, & Sarnak, 1998). Biomarkers are urgently needed to identify patients who are at high-risk for adverse events in order to effectively implement current treatments and also to identify potential candidates for clinical trials of emerging therapeutics.

Serum amyloid A (SAA), a potent inflammatory mediator, is a promising candidate biomarker that has a biologically-plausible mechanistic role in DKD (Anderberg, Meek, Hudkins, et al., 2015). In a cross-sectional study of people with diabetes, serum levels of SAA correlated inversely with estimated glomerular filtration rate (eGFR) and were higher in patients with DKD characterized by severely increased albuminuria (“macroalbuminuria”) compared to those with diabetes and no increase in albuminuria or healthy controls (Anderberg et al., 2015). These findings in humans have been replicated in mouse models of type 1 diabetes (C57/B6 strain with streptozotocin-induced diabetes) and type 2 diabetes (BTBR ob/ob) (Anderberg et al., 2015). In addition to higher circulating SAA levels, greater amounts of SAA mRNA and protein are found in the kidneys of patients with DKD and these corresponding mouse models. Furthermore, SAA is produced by glomerular cells, including both podocytes and mesangial cells, where it induces inflammatory responses and cell death (Meek, LeBoeuf, Saha, et al., 2013).

The aim of the present study was to determine if serum levels of SAA predict actual clinical events of death and ESRD in a longitudinal cohort of study participants with DKD.

2. Materials and methods

2.1. Study population

Study participants were part of a cohort of patients with type 2 diabetes and established DKD enrolled in a prospective, longitudinal observational study focused on coronary artery calcification, the Goldenstate study (Chiu et al., 2010; Mehrotra, Budoff, Christenson, et al., 2004). The participants were recruited from two public hospitals in Los Angeles County, California in the years 2004 through 2008. Type 2 diabetes was defined as a diagnosis of diabetes at ≥30 years of age or treatment with oral hypoglycemic agents or diet for at least 6 months. Criteria for defining DKD were based upon the National Institute of Diabetes and Digestive and Kidney Diseases-sponsored Family Investigation of Nephropathy in Diabetes study: a urine protein-to-creatinine ratio ≥ 500 mg/g at time of enrollment or within the

preceding 12 months and at least one of the following: 1) histological changes of DKD on kidney biopsy, 2) diabetes duration of ≥5 years and a diagnosis of diabetic retinopathy, 3) diabetes duration of ≥10 years without diagnosis of diabetic retinopathy (Knowler, Coresh, Elston, et al., 2005). Of 170 participants enrolled in the original cohort, stored serum samples were available for 141 of them. The final sample included 135 participants after excluding 6 participants for either serum sample integrity or missing clinical data (Fig. 1). The Institutional Review Board at the Los Angeles Biomedical Research Institute and the Providence Health Care Institutional Review Board approved use of data and samples for this study.

2.2. Outcomes assessment

The study participants were evaluated at study visits clinic 12 and 24 months after the baseline visit or within 3 months after reaching ESRD, which was defined as initiation of maintenance dialysis or kidney transplantation. Participants were followed through 31 December 2007, death, or the date of last ESRD data available from the United States Renal Data System or the National Death Index, whichever was later. To establish progression to ESRD or death, participants or their next of kin were contacted every 6 months by telephone. In the case of unsuccessful contact, two certified letters were sent and a study staff member visited the home. Date of dialysis initiation was verified by the United States Renal Data System. Participants who developed ESRD were also followed until death which was verified using the National Death Index.

2.3. Measurements

Body mass index (BMI) was calculated using the formula: weight (kg)/height (m^2). Glycated hemoglobin (HbA_1C) was measured using high performance liquid chromatography (Toshio Medics, Inc., Foster City, CA). Serum creatinine was measured using an enzymatic assay and urine albumin was measured using immunoturbidimetric assays (Kearney, Mount, Watts, Slavin, & Kind, 1987). Urine creatinine was measured using a modified Jaffe reaction, and urine albumin was measured using pyrogallol red (O'Leary, Pembroke, & Duggan, 1992). The eGFR was calculated by the CKD-EPI equation (Matsushita, Mahmoodi, et al., 2012; Matsushita, Tonelli, et al., 2012). Urine albumin-to-creatinine ratio (UACR) was estimated in units of mg/g from a single spot urine collection.

Serum SAA was measured in the year 2015 from serum samples that had been stored at −70 °C until thawed at 4 °C for this assay. Insoluble materials were removed by centrifugation for 10 min at 1000 ×*g* prior to conducting assays. Serum SAA was measured by an enzyme-linked immunosorbent assay for human SAA isoform 1 (www.hycultbiotech.com). Samples were analyzed in duplicate on a 96-well plate, and the colorimetric product was quantified by absorbance at 460 nm on an absorbance plate reader (Tecan Group, Männedorf, Switzerland). Concentrations were calculated by comparison to standard curve generated on each plate. The coefficient of variation was independently verified for the present analyses with inter-and intra-assay coefficients of variation of 10% and 3%, respectively, in samples from study participants.

2.4. Statistical analysis

Baseline characteristics of the study population were calculated by means ± standard deviations for normally distributed variables and medians, interquartile ranges for non-normally distributed variables, and percentages for categorical variables. Parametric and nonparametric analysis of variance models were used for group comparisons at baseline for normally and non-normally distributed continuous data, respectively. Chi-square tests were performed for categorical data.

Serum levels of SAA were examined as the main predictor along with pre-specified covariates for multivariable analyses. The pre-specified covariates were established risk factors for DKD including: age, sex, race/ethnicity, diabetes duration, blood pressure, BMI, glycemic control (HbA_1C), renin–angiotensin–aldosterone system (RAAS) inhibition (use of angtiotensin converting enzyme inhibitors or angiotensin receptor blockers), UACR, and eGFR (Earle & Viberti, 1994; Forsblom, Moran, Harjutsalo, et al., 2014; Pavkov, Knowler, Hanson, Bennett, & Nelson, 2008). Units of the covariates were analyzed and reported in clinically-meaningful units (e.g. eGFR in 10 ml/min/1.73 m^2, age in decades, diabetes duration in 5 year increments, systolic and diastolic blood pressure in 10 mmHg and 5 mmHg increments, respectively). When UACR and SAA were analyzed as continuous variables, they were log-transformed due to data skewness and back converted for reporting.

The primary outcome was the composite of death and ESRD events. Secondary outcomes were individual outcomes of death or ESRD. Unadjusted incidence rates were calculated by determining the number of incidence per 100-person years. Cox proportional hazards regression was used to estimate the relative hazards of the primary outcome and secondary outcomes. Nested models were analyzed for each of the primary and secondary outcomes with the base model including eGFR and UACR as predictors (Model 0). In the following models, predictor variables were sequentially added: SAA tertiles (Model 1); sex, race/ethnicity, and age (Model 2); and RAAS inhibition, HbA1c, diabetes duration, BMI, systolic blood pressure, and diastolic blood pressure (Model 3) (Matsushita, Tonelli, et al., 2012). Kaplan–Meier survival curves were used to evaluate associations of SAA by tertile with the primary and secondary outcomes.

To provide internal validation for estimates of hazard ratios and confidence intervals, bootstrap resampling was utilized (Matsushita, Tonelli, et al., 2012). Cox proportional hazards regression was conducted on Models 0–3 with predictor variables as described above. Cox proportional models were also used to estimate the relative hazard for SAA as a continuous variable for the same models. Fine-Gray proportional hazards models were used to assess potential competing risk for death in the secondary outcome of ESRD (Fine & Gray, 1999).

Receiver operation characteristic (ROC) curves and C statistics were generated to determine how addition of SAA improved predictive utility of the models. Based on 100,000 bootstrapped statistics, 95% confidence intervals (CIs) for delta C statistics were generated (Fufaa, Weil, Nelson, et al., 2015). Model over-fitting was minimized by limiting the number of covariates and using a sequential approach in the Cox models (Models 0–3). Multi-collinearity was checked using variance inflation factor, and model fit was evaluated using

Akaike's information criterion (AIC). Statistical analyses were conducted with R version 3.1.2 for Windows. The threshold for statistical significance for all analyses was set at an alpha level of $p < 0.05$.

3. Results

3.1. Participant characteristics

The median follow-up time was 3.5 years. Study participants (n = 135) had an age of 57.2 ± 7.2 years and diabetes duration of 15.1 ± 6.1 years (Table 1). The participants were 55% men (75/135) and 73% Mexican-American (99/135). They had a systolic blood pressure of 153 ± 27.7 mm Hg, body mass index of 31 ± 9 kg/m^2, and HbA1c of 8.6 ± 2.4%. Their mean eGFR was 55.5 ± 22.3 ml/min/1.73 m^2, and median (interquartile range) UACR was 1861 (720–3912) mg/g. RAAS inhibitors were taken by 78% (105/135) of participants: 64% (87/135) took angiotensin converting enzyme inhibitors, 23% (31/135) took angiotensin receptor blockers, and 9% (12/135) took both.

When stratified by tertiles for serum levels of SAA (tertile 1: SAA ≤0.55 μg/ml; tertile 2: 0.55 < SAA ≤1.0 μg/ml; tertile 3: SAA > 1.0 μg/ml), there were no differences in most clinical characteristics among the groups. However, a higher proportion of Mexican-Americans were present in SAA tertile 2 (40/45) compared to SAA tertile 3 (23/45), and a higher proportion of women were present in SAA tertile 3 (25/45) compared to SAA tertile 1 (15/45).

3.2. Primary outcome of death and ESRD

Unadjusted incidence rates for the primary composite outcome (death and ESRD) were 9.7, 14.5, and 22.3 events per 100 person-years for SAA tertiles 1, 2, and 3, respectively (Table 2). In the Cox regression model adjusted for eGFR, UACR, and SAA (Model 1), participants in SAA tertile 3 had a hazard ratio (HR) for the primary outcome of 2.67 (95% CI 1.37–5.25, p = 0.004; Table 3) compared to SAA tertile 1. These associations strengthened to an HR of 3.03 (95% CI 1.43–6.40, p = 0.003) with additional adjustments for age, sex, and race/ethnicity (Model 2). The associations strengthened even more with adjustment for RAAS inhibition, HbA1c, diabetes duration, BMI, systolic blood pressure, and diastolic blood pressure (Model 3, Supplemental Table 2). Differences in survival probabilities for death and ESRD were found between SAA tertiles, with 23% (10/45) and 47% (21/45) of participants reaching the primary outcome by 3 years in SAA tertiles 1 and 3, respectively (Fig. 2A).

3.3. Secondary outcome of death

The unadjusted incidence rates for the secondary outcome of death were 4.0, 2.6, and 17.2 events per 100 person-years for SAA tertiles 1, 2, and 3, respectively (Table 2). In the Cox regression model adjusted for eGFR, UACR, and SAA (Model 1), participants with SAA levels in tertile 3 had an HR for death of 4.74 (95% CI 2.00–11.46, p < 0.001; Table 3) compared to SAA tertile 1. The associations strengthened with additional adjustments for age, sex, and race/ ethnicity (Model 2) with an HR of 7.42 (95% CI 2.56–21.51, p < 0.001). They remained similar after adjustment for RAAS inhibition, HbA1c, diabetes duration,

BMI, systolic blood pressure, and diastolic blood pressure (Model 3, Supplemental Table 2). Differences in survival probabilities were found between tertiles with 11% (5/45) and 40% (18/45) of participants reaching the death outcome by 3 years in SAA tertiles 1 and 3, respectively (Fig. 2B).

3.4. Secondary outcome of end-stage renal disease

The unadjusted incidence rates for the secondary outcome of ESRD were 6.8, 12.3, and 10.0 events per 100 person-years for SAA tertiles 1, 2, and 3, respectively (Table 2). In the Cox regression model adjusted for eGFR, UACR, and SAA (Model 1), participants in SAA tertile 3 had an HR for ESRD of 1.82 (95% CI 0.75–4.47, p = 0.2; Table 3) compared to SAA tertile 1. With additional adjustments for age, sex, and race/ ethnicity (Model 2), the HR was 1.82 (95% CI 0.68–4.89, p = 0.2). Further adjustment for RAAS inhibition, HbA1c, diabetes duration, BMI, systolic blood pressure, and diastolic blood pressure did not modify these associations (Model 3, Supplemental Table 2). However, when death was examined as a competing risk for ESRD, SAA tertile 3 had an HR of 2.74 (95% CI 1.21–6.21, p = 0.02) compared to tertile 1 for the ESRD outcome. Differences in survival probabilities for ESRD events after 3 years between SAA tertiles 1 and 3 were 16% (7/45) and 32% (14/45), respectively (Fig. 2C).

3.5. Validation by bootstrap resampling and SAA as a continuous predictor variable

With bootstrapping for 500 observations, associations of higher SAA tertiles with death and ESRD as the composite primary outcome and individual outcomes were confirmed and strengthened (Table 4, Supplemental Table 3) (Ju, Nair, Smith, et al., 2015). Notably, SAA tertile 3 had an HR of 2.49 (95% CI 1.50–4.14, p < 0.001) and SAA tertile 2 had an HR of 1.67 (95% CI 1.08–2.59, p = 0.02) for ESRD compared to SAA tertile 1. Similarly, SAA examined as a continuous predictor variable produced results congruent with those in the models with SAA stratified by tertiles (Supplemental Table 4).

3.6. Comparative model fit indices

The ROC analysis demonstrated that inclusion of SAA improved the model *C-statistic* ($\Delta c = 0.017$) for the primary outcome. Analysis using AIC confirmed the ROC findings that included SAA in the models for predicting risk of the primary outcome (AIC = 483 in model with SAA; AIC = 490 in model without SAA). Using multi-collinearity diagnostics, the maximum variance inflation factor among the variables was 1.33, indicating that multi-collinearity was minimal (Supplemental Table 5).

4. Discussion

High levels of serum SAA predicted significantly increased risk of the primary outcome of death and ESRD in patients with DKD. Levels of SAA >1.0 μg/ml versus <0.55 μg/ml were associated with hazards of the primary outcome of approximately 3-fold and of death per se by more than 7-fold. Notably, SAA was the single strongest predictor for death of all variables tested across various models. SAA improved risk prediction beyond established DKD risk factors as evidenced by improved indices of model discrimination.

These longitudinal data are the first to reveal the utility of SAA to improve risk prediction for death and ESRD in patients with DKD. The previous cross-sectional findings of associations of SAA with albuminuria (positive) and eGFR (inverse) have been now extended to predict risk of actual clinical events in DKD (Anderberg, et al., 2015; Kumon, Suehiro, Itahara, Ikeda, & Hashimoto, 1994). The present study was conducted in a largely Mexican-American cohort of individuals with type 2 diabetes and prevalent DKD. A recent longitudinal cohort study of Scandinavian-Europeans with type 1 diabetes reported that higher circulating levels of SAA predicted new cases of albuminuria, an indicator of DKD onset, and was an even more robust predictor than established risk factors (Overgaard et al., 2013). Collectively, these observations suggest that SAA predicts incident DKD, as well as adverse outcomes of prevalent DKD, and that it has relevance for types 1 and 2 diabetes in different racial/ethnic groups.

An important finding in this study was the degree to which comparative model fit indices showed that SAA improved DKD risk prediction. To put these effect sizes in perspective, a recent study designed to validate emerging DKD biomarkers, including neutrophil gelatinase-associated lipocalin, reported smaller improvement in risk prediction for ESRD (Δ *C-statistic* 0.005) or death (Δ *C-statistic* 0.006) and were considered clinically relevant (Fufaa et al., 2015). By comparison, SAA would be considered relevant and to have substantial potential as a biomarker for risk-stratification in patients with DKD.

SAA is a biologically-plausible mediator and targetable biomarker for DKD. It is a potent pro-inflammatory protein that is increased in the blood and the kidneys of patients with DKD. SAA is not increased in the blood or kidneys of patients with non-DKD or in individuals with diabetes and no kidney disease (Anderberg et al., 2015). SAA deposition without amyloidosis is unique to DKD. No immunostaining for SAA was present in kidneys from control patients, and Congo red staining for amyloidosis was negative in all kidneys tested (DKD and non-DKD) (Anderberg et al., 2015). SAA is produced locally in the glomerular and tubulointerstitial compartments of the kidney. SAA was increased at both the mRNA and protein levels in glomerular and tubular cells of diabetic mouse models and in kidney biopsy samples from patients with DKD. Exposure to diabetic-like conditions, in particular advanced glycation end products, has been shown to cause marked increase in production of SAA by podocytes and mesangial cells (Meek et al., 2013). SAA activated these cells to produce even more SAA, suggesting that it may act in a feed-forward loop to perpetuate up-regulation of inflammation (Anderberg et al., 2015). SAA also reduces the anti-inflammatory function of high-density lipoprotein cholesterol (Han, Subramanian, Chan, et al., 2007). Patients with early-to-late stage chronic kidney disease have elevated levels of high-density-lipoprotein-bound SAA that amplifies tissue inflammation (Kisilevsky & Subrahmanyan, 1992; Shoji, Emoto, Kawagishi, et al., 2001; Tape & Kisilevsky, 1990). Therefore, SAA is likely to be a link between aberrant metabolic products and inflammatory responses that lead to diabetic complications including DKD.

This study has both limitations and strengths. First, only baseline data regarding study participant risk factors for DKD were available. Future studies with longitudinal assessments of risk factors and treatments in patients with DKD will more clearly delineate independent risks associated with higher SAA levels over time. Second, the study cohort had a high

proportion of patients with Mexican-American heritage. This is a strength of the present report because this high-risk ethnic group has been under-represented in previous studies of DKD. Nevertheless, the distribution of race/ethnicity and sex varied slightly among SAA tertiles, and although these variables were controlled in multiple-variable models, studies of more diverse groups are needed to assure generalizability. Third, while a signal for increased risk of ESRD was observed when death was examined as a competing risk for ESRD and in bootstrap resampling, larger studies are required to verify that SAA predicts ESRD per se. Fourth, confidence intervals for some hazards ratios were comparatively wide, indicating an element of imprecision in corresponding effect sizes. Finally, patients who did not enter treatment for ESRD or who died outside the United States would not have been captured by linkage to United States Renal Data System or the National Death Index. Although this scenario is possible, it is likely rare. Furthermore, this sort of loss would only weaken the association of SAA to outcome events, and therefore, make these estimates conservative.

In conclusion, high serum levels of SAA predicted increased risk for death and ESRD in a cohort of study participants with DKD. SAA added to risk estimation beyond that predicted by established DKD risk factors. SAA is a promising targetable biomarker for DKD and may prove useful in identifying patients for studies of novel anti-inflammatory therapies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jdiacomp.2016.07.018.

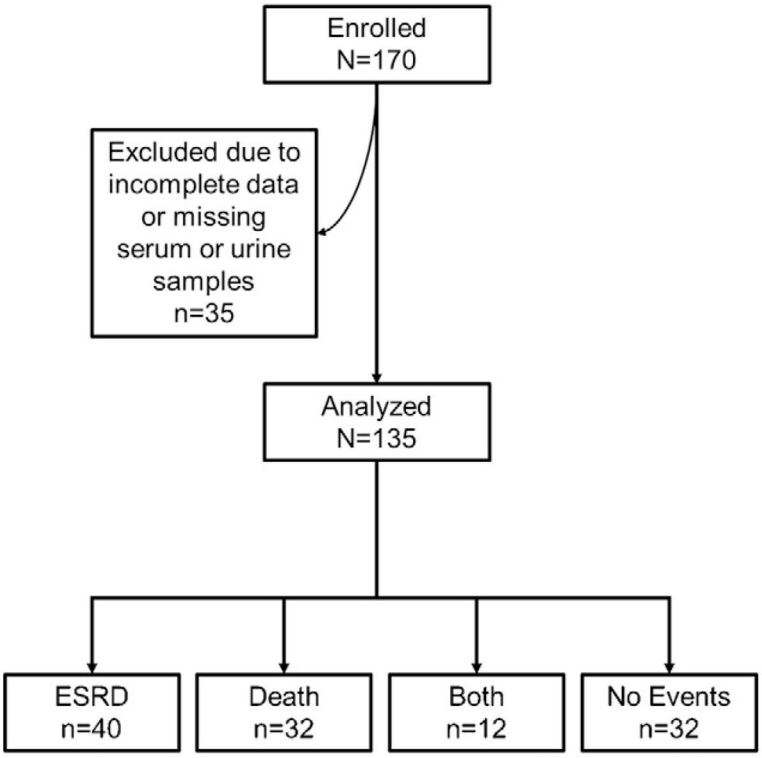

Fig. 1.
CONSORT diagram of study participants.

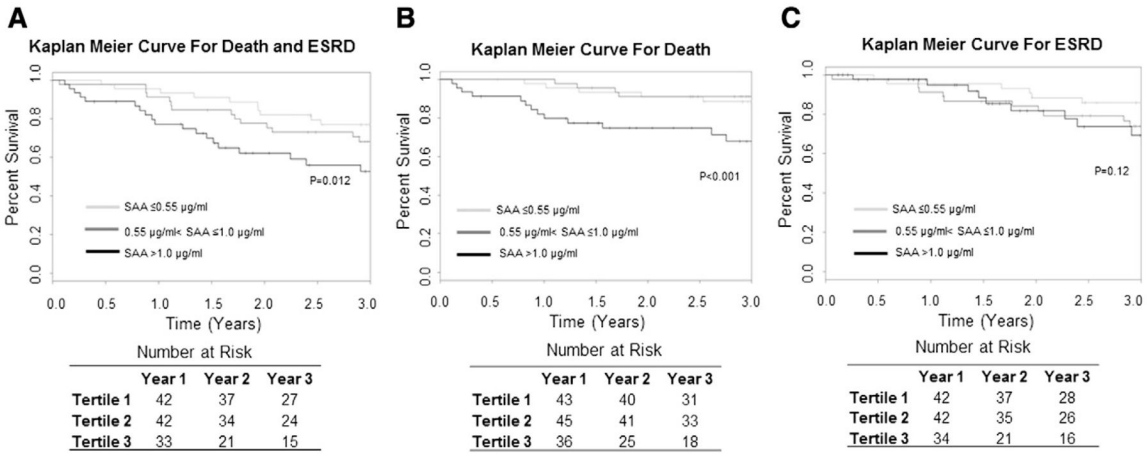

Fig. 2.

Kaplan–Meier survival curves by tertiles of serum SAA for A) primary outcome of death and ESRD, B) secondary outcome of death, and C) secondary outcome of ESRD.

Table 1

Baseline demographics and characteristics of study participants stratified by tertile of serum SAA.

	Overall	SAA Tertile 1 (n = 45)	SAA Tertile 2 (n = 45)	P-value	SAA Tertile 3 (n = 45)	P-value
N	135	45	45		45	
Age (y)	57.2 (7.5)	57.6 (8.3)	57.6 (6.5)	0.9	56.6 (7.6)	0.8
Sex	75 M 60 F	30 M 15 F	25 M 20 F	0.4	20 M 25 F	0.2
Race/ethnicity				0.3		0.01
African-American	12 (9%)	5 (11%)	1 (2%)		6 (13%)	
Mexican-American	99 (73%)	36 (80%)	40 (89%)		23 (51%)	
Other	24 (18%)	4 (9%)	4 (9%)		16 (36%)	
Systolic blood pressure (mm Hg)	153.3 (27.7)	155.3 (28.7)	154.7 (27.6)	0.9	150.1 (27.0)	0.6
Diastolic blood pressure (mm Hg)	76.7 (13.2)	77.9 (13.7)	75.5 (12.6)	0.7	76.6(13.5)	0.9
RAAS inhibition	78% (105/135)	76% (34/45)	80% (36/45)	0.8	78% (35/45)	0.9
Diabetes duration (y)	15.1 (6.1)	15.4 (6.1)	15.8 (6.4)	0.9	14.1 (5.9)	0.6
HbA1c (%)	8.6 (2.3)	8.6 (1.9)	8.5 (2.3)	0.9	8.8 (2.6)	0.9
BMI (kg/m^2)	31 (9)	29 (7)	30 (7)	0.9	34.5 (11.1)	0.01
eGFR (ml/min/1.73 m^2)	56 (22)	62 (22)	51 (20)	0.04	55.0 (23.7)	0.3
UACR (mg/g)	1861 (720–3912)	1842 (751–3646)	2107 (813–4152)	0.7	1123 (530–3389)	0.4

Participants were stratified by tertiles of serum concentrations of SAA with cut-points to divide SAA into tertiles; Tertile 1: SAA ≤ 0.55 μg/ml, Tertile 2: 0.55 < SAA ≤1.0 μg/ml; Tertile 3: SAA > 1.0 μg/ml. P values are for comparison of tertile 1 against tertile 2 and tertile 3, respectively. Values are presented as mean (standard deviation, SD) or median (interquartile range, IQR). BMI: Body mass index; eGFR: estimated glomerular filtration rate by serum creatinine CKD-EPI calculation, HbA1c: glycated hemoglobin, RAAS: Renin-angiotensin-aldosterone-system, UACR: Urine albumin-to-creatinine ratio.

Table 2

Unadjusted incidence rates stratified by tertile of SAA.

	Death and ESRD		Death		ESRD	
	Events	**Unadjusted Incidence Rates (per 100 person-years)**	**Events**	**Unadjusted Incidence Rates (per 100 person-years)**	**Events**	**Unadjusted Incidence Rates (per 100 person-years)**
Tertile 1 (SAA ≤ 0.55 μg/ml)	20	9.7	8	4.0	12	6.8
Tertile 2 (0.55 < SAA ≤ 1.0 μg/ml)	23	14.5	5	2.6	18	12.3
Tertile 3 (SAA > 1.0 μg/ml)	29	22.3	19	17.2	10	10.0

Table 3

Hazard ratios (HR) and 95% confidence intervals for tertiles of serum SAA.

		Primary Outcome Death and ESRD				**Death Outcome**				**ESRD Outcome**			
Model 0	Variable	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**
	eGFR (ml/min/1.73 m^2)	**0.97**	**0.95**	**0.98**	**<0.001**	**0.98**	**0.96**	**0.99**	**0.01**	**0.95**	**0.93**	**0.97**	**<0.001**
	UACR (mg/g)	**2.69**	**1.38**	**5.24**	**0.01**	1.02	0.48	2.18	0.9	**4.54**	**1.79**	**11.55**	**0.001**
Model 1	Variable	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**
	SAA Tertile 2 (0.55 < SAA ≤1.0 μg/ml)	1.02	0.52	2.02	0.9	0.44	0.14	1.39	0.2	1.10	0.51	2.40	0.8
	SAA Tertile 3 (SAA > 1.0 μg/ml)	**2.67**	**1.37**	**5.25**	**0.004**	**4.74**	**2.00**	**11.46**	**<0.001**	1.82	0.75	4.42	0.2
	eGFR (10 ml/min/1.73 m^2)	**0.72**	**0.61**	**0.84**	**<0.001**	**0.77**	**0.63**	**0.93**	**0.008**	**0.62**	**0.49**	**0.77**	**<0.001**
	Log UACR (mg/g)	**1.28**	**1.15**	**1.43**	**<0.001**	**1.20**	**1.03**	**1.38**	**0.02**	**1.30**	**1.14**	**1.49**	**<0.001**
Model 2	Variable	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**
	SAA Tertile 2 (0.55 < SAA ≤1.0 μg/ml)	1.02	0.50	2.09	0.9	0.62	0.19	2.02	0.4	0.94	0.41	2.15	0.9
	SAA Tertile 3 (SAA > 1.0 μg/ml)	**3.03**	**1.43**	**6.40**	**0.003**	**7.42**	**2.56**	**21.51**	**<0.001**	1.82	0.68	4.89	0.2
	eGFR (10 ml/min/1.73 m^2)	**0.70**	**0.59**	**0.83**	**<0.001**	**0.79**	**0.64**	**0.97**	**0.02**	**0.59**	**0.46**	**0.74**	**<0.001**
	Log UACR (mg/g)	**1.28**	**1.15**	**1.42**	**<0.001**	**1.22**	**1.05**	**1.40**	**0.01**	**1.30**	**1.13**	**1.50**	**0.001**
	Race (Mexican-American)	1.34	0.62	2.88	0.5	0.83	0.33	2.10	0.7	1.82	0.61	5.47	0.3
	Sex (Male)	1.15	0.66	2.02	0.6	2.68	0.23	5.86	0.01	0.72	0.36	1.43	0.3
	Age (Per Decade)	1.01	0.67	1.52	0.9	1.51	0.83	2.75	0.2	0.76	0.44	1.29	0.6

Model 0 included eGFR and UACR.

Model 1 included eGFR, UACR, and the 2nd and 3rd tertiles of SAA as a predictor with the 1st tertile as the reference category.

Model 2 included eGFR, UACR, race, sex, age, and the 2nd and 3rd tertiles of SAA as a predictor with the 1st tertile as the reference category. eGFR: estimated glomerular filtration rate, UACR: Urine albumin-to-creatinine ratio.

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Table 4

SAA as tertile variable with bootstrap resampling for 500 observations.

		Primary Outcome Death and ESRD				**Death Outcome**				**ESRD Outcome**			
Model 0	Variable	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**
	eGFR (ml/min/1.73 m^2)	**0.96**	**0.95**	**0.97**	**<0.001**	**0.95**	**0.94**	**0.96**	**<0.001**	**0.95**	**0.94**	**0.96**	**<0.001**
	UACR (mg/g)	**1.46**	**1.325**	**1.71**	**<0.001**	1.07	0.90	1.28	<0.001	**1.93**	**1.55**	**2.39**	**<0.001**
Model 1	Variable	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**
	SAA Tertile 2 (0.55 < SAA ≤ 1.0 μg/ml)	1.40	0.99	1.99	0.06	0.90	0.51	1.59	0.7	**1.70**	**1.13**	**2.54**	**0.01**
	SAA Tertile 3 (SAA > 1.0 μg/ml)	**3.14**	**2.20**	**4.47**	**<0.001**	**6.35**	**3.86**	**10.44**	**<0.001**	**2.45**	**1.56**	**3.87**	**<0.001**
	eGFR (10 ml/min/1.73 m^2)	**0.74**	**0.68**	**0.81**	**<0.001**	**0.76**	**0.69**	**0.84**	**<0.001**	**0.59**	**0.52**	**0.66**	**<0.001**
	Log UACR (mg/g)	**1.53**	**1.32**	**1.79**	**<0.001**	**1.22**	**1.03**	**1.44**	**0.02**	**1.93**	**1.58**	**2.38**	**<0.001**
Model 2	Variable	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**	**HR**	**Lower**	**Upper**	**P-value**
	SAA Tertile 2 (0.55 < SAA ≤ 1.0 μg/ml)	**1.49**	**1.03**	**2.14**	**0.03**	1.25	0.69	2.25	0.5	**1.67**	**1.08**	**2.59**	**0.02**
	SAA Tertile 3 (SAA > 1.0 μg/ml)	**3.17**	**2.15**	**4.66**	**<0.001**	**13.85**	**7.47**	**25.66**	**<0.001**	**2.49**	**1.50**	**4.12**	**<0.001**
	eGFR (10 ml/min/1.73 m^2)	**0.75**	**0.69**	**0.82**	**<0.001**	**0.78**	**0.70**	**0.86**	**<0.001**	**0.56**	**0.49**	**0.63**	**<0.001**
	Log UACR (mg/g)	**1.58**	**1.35**	**1.85**	**<0.001**	**1.26**	**1.04**	**1.53**	**0.02**	**1.96**	**1.58**	**2.44**	**<0.001**
	Race (Mexican-American)	1.00	0.66	1.51	0.9	1.30	0.79	2.14	0.30	**1.95**	**1.10**	**3.45**	**0.02**
	Sex (Male)	0.98	0.73	1.31	0.9	**2.76**	**1.86**	**4.10**	**<0.001**	**0.68**	**0.47**	**0.98**	**0.04**
	Age (Per Decade)	1.06	0.85	1.33	0.6	**1.95**	**1.47**	**2.59**	**<0.001**	0.81	0.61	1.06	0.1

Model 0 included eGFR and UACR.

Model 1 included eGFR, UACR, and the 2nd and 3rd tertiles of SAA as a predictor with the 1st tertile as the reference category.

Model 2 included eGFR, UACR, race, sex, age, and the 2nd and 3rd tertiles of SAA as a predictor with the 1st tertile as the reference category. eGFR: estimated glomerular filtration rate, UACR: Urine albumin-to-creatinine ratio.