






Association of Multiple Plasma Biomarker Concentrations with Progression of Prevalent Diabetic Kidney Disease: Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study

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on behalf of the CKD Biomarkers Consortium and the Chronic Renal Insufficiency Cohort (CRIC) Study Investigators*

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ABSTRACT

Background Although diabetic kidney disease is the leading cause of ESKD in the United States, identifying those patients who progress to ESKD is difficult. Efforts are under way to determine if plasma biomarkers can help identify these high-risk individuals.

Methods In our case-cohort study of 894 Chronic Renal Insufficiency Cohort Study participants with diabetes and an eGFR of <60 ml/min per 1.73 m² at baseline, participants were randomly selected for the subcohort; cases were those patients who developed progressive diabetic kidney disease (ESKD or 40% eGFR decline). Using a multiplex system, we assayed plasma biomarkers related to tubular injury, inflammation, and fibrosis (KIM-1, TNFR-1, TNFR-2, MCP-1, suPAR, and YKL-40). Weighted Cox regression models related biomarkers to progression of diabetic kidney disease, and mixed-effects models estimated biomarker relationships with rate of eGFR change.

Results Median follow-up was 8.7 years. Higher concentrations of KIM-1, TNFR-1, TNFR-2, MCP-1, suPAR, and YKL-40 were each associated with a greater risk of progression of diabetic kidney disease, even after adjustment for established clinical risk factors. After accounting for competing biomarkers, KIM-1, TNFR-2, and YKL-40 remained associated with progression of diabetic kidney disease; TNFR-2 had the highest risk (adjusted hazard ratio, 1.61; 95% CI, 1.15 to 2.26). KIM-1, TNFR-1, TNFR-2, and YKL-40 were associated with rate of eGFR decline.

Conclusions Higher plasma levels of KIM-1, TNFR-1, TNFR-2, MCP-1, suPAR, and YKL-40 were associated with increased risk of progression of diabetic kidney disease; TNFR-2 had the highest risk after accounting for the other biomarkers. These findings validate previous literature on TNFR-1, TNFR-2, and KIM-1 in patients with prevalent CKD and provide new insights into the influence of suPAR and YKL-40 as plasma biomarkers that require validation.

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Diabetic kidney disease (DKD) develops in approximately 30% of individuals with type 1 diabetes mellitus and 40% of individuals with type 2 diabetes

mellitus.¹ DKD is the leading cause of ESKD in the United States.² Pathways and processes leading to progression of DKD are heterogeneous,³ including

inflammation, fibrosis, and tubular injury, which makes it difficult to identify patients who will develop DKD progression, and ultimately ESKD. These diverse mechanisms may also contribute to the variability in response to interventions aimed at slowing disease progression.

To date, the established diagnostic and prognostic tools for DKD have limitations. A kidney biopsy is the reference standard for diagnosis, but it is invasive and associated with risk, and provides glomerular and tubulointerstitial histology of modest prognostic value. The best measure of kidney function is eGFR, which carries prognostic information, but is limited due to unreliability.^{4,5} Albuminuria has also been shown to strongly predict DKD progression, but many individuals with diabetes develop regression of albuminuria.^{6–8} Furthermore, pathologic lesions^{3,9,10} and DKD progression occur in the absence or presence of low levels of albuminuria.^{11–14} Thus, there is an unmet need for discovering better biomarkers that can identify individuals at high risk of progression of DKD who may benefit from more intensified or targeted treatments.

In the past decade, investigations have elucidated specific DKD progression pathways, along with identification of associated biomarkers.^{15,16} Recent studies have reported associations of both the initiation and the progression of DKD with several proinflammatory and profibrotic plasma markers, such as soluble TNF receptors 1 and 2 (TNFR-1 and TNFR-2),^{17–21} YKL-40 (a heparin- and chitin-binding glycoprotein),²² monocyte chemotactic protein-1 (MCP-1, also known as the C-C chemokine ligand 2),²³ and soluble urokinase-type plasminogen activator receptor (suPAR),²⁴ and biomarkers of tubular injury, including kidney injury molecule 1 (KIM-1).^{25,26} Prior studies of DKD progression have been limited by relatively small samples, especially of individuals with already established type 2 diabetes, and including those with both normal and impaired kidney function, reporting only one or two biomarker associations, and without determining

Significance Statement

In diabetic kidney disease, ascertaining which patients will progress to ESKD is difficult. Efforts are under way to determine whether plasma biomarkers can identify these high-risk individuals; such biomarkers may inform development of therapies and selection of individuals for clinical trials. In this case-cohort study of well-phenotyped individuals with diabetic kidney disease, increased concentrations of plasma biomarkers related to tubular injury, inflammation, and fibrosis (kidney injury molecule 1 [KIM-1], TNF receptor 1 [TNFR-1], TNFR-2, monocyte chemotactic protein-1, soluble urokinase-type plasminogen activator receptor [suPAR], and YKL-40) were associated with increased risk of progression of diabetic kidney disease. After accounting for the other biomarkers, higher TNFR-2 levels were most strongly associated with disease progression. These findings validate the previous literature on TNFR-1, TNFR-2, and KIM-1, and provide new insights on suPAR and YKL-40 as plasma markers of diabetic kidney disease progression that require validation.

inter-relationships between biomarkers. Accordingly, we assessed if plasma biomarkers related to inflammation, fibrosis, and tubular injury were associated with the progression of DKD in the well-characterized subpopulation of individuals with mild to moderate kidney disease and diabetes in the Chronic Renal Insufficiency Cohort (CRIC) Study. We hypothesized that plasma concentrations of KIM-1, TNFR-1, TNFR-2, YKL-40, suPAR, and MCP-1 were associated with DKD progression and eGFR decline over time.

METHODS

Study Design and Study Population

This study uses a case-cohort design of participants with diabetes within the CRIC Study, a multicenter, prospective, observational cohort study of 3939 men and women with mild to moderate CKD in the United States.^{27,28} The case-cohort design was chosen to reduce the need to assay biomarkers in the full cohort, while still retaining the validity to conduct prospective analyses. Briefly, CRIC Study participants were recruited from seven clinical centers from 2003 to 2008. The inclusion and exclusion criteria have been detailed previously.^{27,28} The major eligibility criteria included adults 21–74 years of age with eGFR from 20 to 70 ml/min per 1.73 m². The CRIC Study protocol was approved by the institutional review boards of all participating centers, and is in accordance with the Declaration of Helsinki. All participants provided written informed consent.

CRIC participants were eligible for this study if they had a diagnosis of diabetes (made on the basis of a fasting glucose of ≥ 126 mg/dl, a nonfasting glucose of ≥ 200 mg/dl, or self-reported use of insulin or other medications for glycemic control), an eGFR of < 60 ml/min per 1.73 m², and if their plasma biospecimens had been collected ($n = 1315$). The baseline for each participant was defined as the annual study visit at which the plasma samples were collected. The time between the

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CRIC baseline visit, at which covariate data were collected, and the visit at which plasma samples were collected was approximately 1 year (median, 370 days; interquartile range [IQR], 0–713 days). A subcohort was constructed as a randomly selected subset of all eligible participants, independent of case status. In addition, all of the individuals who developed the primary outcome of interest, defined as ESKD or 40% decline in baseline eGFR, were included in the study population as cases (see Supplemental Figure 1 for study flow diagram).

Plasma Biomarker Measurements

Plasma biomarker concentrations were the primary exposure in this study. Plasma samples were collected at baseline (2003–2010) and stored at -80°C until they were thawed for measurement of the biomarkers KIM-1, MCP-1, TNFR-1, TNFR-2, suPAR, and YKL-40, using a multiplex U-PLEX assay on the Meso Scale Discovery platform (Meso Scale Discovery, Gaithersburg, MD). All of the following antibodies (capture and detection antibodies) were obtained from R&D Systems (Bio-Techne): KIM-1 (catalog numbers AF1750 and AF1750); YKL-40 (catalog numbers MAB25991 and AF2577); TNFR-1 (catalog numbers MAB625 and MAB225); TNFR-2 (catalog numbers MAB726 and AF726); suPAR (catalog numbers MAB807 and AF807); MCP-1 (catalog numbers MAB679 and AF279). The biomarkers were selected on the basis of prior published reports and preliminary data generated by the CKD Biomarker Consortium investigators.^{18,24,26,29–31} Biomarker concentrations were expressed as the mean of duplicate measurements. Overall, intra-assay coefficients of variation were all $\leq 7\%$, and interassay coefficients of variation were $< 15.3\%$. Please see Supplemental Table 1 for quality-control parameters for each biomarker assay. All assays were performed blinded to clinical outcomes.

Other Study Variables

Information on age, sex, race/ethnicity, education, and medical history (including hypertension, prevalent cardiovascular disease, smoking status, and medication use) were collected through self-report. Using a standardized technique, height, weight, and BP were measured. BP was measured in triplicate, and the mean of the latter two measurements was recorded. Hypertension was defined by a systolic BP > 140 mm Hg, a diastolic BP > 90 mm Hg, or self-reported use of antihypertensive medications.³² Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Blood samples were used to measure hemoglobin A1c, serum high-sensitivity C-reactive protein (hsCRP), and serum creatinine and cystatin C concentrations for the estimation of GFR with a CRIC-specific equation.³³ Proteinuria was assessed using assays of urine collections of creatinine and protein (urine protein-creatinine ratio [UPCR] in milligrams per gram).

Study Outcomes

The primary outcome of interest, DKD progression, was defined as time to incident ESKD (determined through self-report,

review of medical records, and linkage to the US Renal Data System) or a $\geq 40\%$ decline (relative to baseline) during the follow-up period. A secondary outcome of interest included the yearly change in eGFR over time in milliliters per minute per 1.73 m^2 . Study participants were followed until the occurrence of the primary end point, death, withdrawal from the study, or the end of the follow-up period (mid-2017).

Statistical Analyses

We described the study population using mean (SD) or median (IQR) for continuous variables, and frequency and proportion for categorical variables. The Pearson chi-squared or Fisher exact test and ANOVA or Kruskal–Wallis test were used to compare categorical and continuous variables, respectively. Partial Pearson pairwise correlations, adjusting for age and sex, were assessed to evaluate the relationship between biomarkers, eGFR, and UPCR.

Multivariable, weighted, Cox proportional hazards models that accounted for the case-cohort design were used to assess the association between each plasma biomarker concentration with DKD progression. A weighted regression was used to account for differential sampling probabilities between the subcohort and additional cases to permit estimation of relative risk and rates of the exposure-outcome relationship.³⁴ We performed hierarchic modeling with sequential adjustment for sociodemographic covariates, including sex, race/ethnicity, education, study site; and clinical characteristics, including systolic BP, diastolic BP, BP medications, smoking status, BMI, hsCRP, and hemoglobin A1c; and then by adding eGFR alone, UPCR alone, and finally adjusting for both eGFR and UPCR. We also assessed the interaction between baseline eGFR (< 45 versus ≥ 45 ml/min per 1.73 m^2) and DKD progression, expecting that those with a lower baseline eGFR to have enhanced biomarker associations with DKD progression.³⁵

As a sensitivity analysis, to address potential batch effects of the biospecimen assays, we used the ComBat approach, which corrects batch differences in mean and variance of the assay values *via* empirical Bayes estimation.³⁶ Further, to assess the potential influence of imbalance between the subcohort and additional cases, we limited the Cox proportional hazards models to the subcohort only to assess the consistency of results from the primary analysis using weighted Cox proportional hazards models. As an exploratory analysis, we also fit a multivariable model to evaluate the interrelationships of the six biomarkers in their association with DKD progression. We used a backward selection algorithm to create a parsimonious model, retaining markers with a significance level of $P < 0.05$.

Mixed-effects models were used to assess the relations of biomarker concentrations with yearly change in eGFR within the subcohort only. All biomarkers were logarithmically transformed to normalize their skewed distributions, and \log_2 transformed variables were used in all regression analyses. Adjusted hazard ratios (aHRs) with 95% confidence intervals were reported. Analyses were performed with SAS 9.4 (SAS

Table 1. Baseline characteristics of CRIC participants with diabetes overall and by case status

Characteristics	Eligible Cohort (n=1315)	Random Subcohort (n=596)	All Cases (n=538)
Age, yr (SD)	61.0 (9.3)	60.8 (9.3)	58.6 (9.8)
Sex, %			
Male	57.2	58.2	60.6
Female	42.8	41.8	39.4
Race/ethnicity, %			
Non-Hispanic White	36.0	37.2	25.8
Non-Hispanic Black	41.2	40.8	45.5
Hispanic	19.2	18.5	25.1
Other	3.7	3.5	3.5
Education, %			
Less than high school	27.2	28.0	31.4
High school and some college	48.4	47.5	48.3
College degree and higher	24.4	24.5	20.3
Smoking status, %			
Current	11.2	11.4	12.3
Former	45.0	43.1	45.2
Never	43.8	45.5	42.6
Hypertension, %	95.4	96.0	96.7
Systolic BP (mm Hg), mean (SD)	133 (24)	133 (24)	140 (24)
Diastolic BP (mm Hg), mean (SD)	68 (13)	68 (12)	71 (13)
Antihypertensive medication use, %	98.4	98.1	98.9
Self-reported cardiovascular disease, %	45.6	43.6	45.9
BMI (kg/m ²), mean (SD)	34.1 (7.8)	33.8 (7.7)	33.9 (8.0)
Hemoglobin A1c, mean (SD)	7.2 (1.5)	7.2 (1.5)	7.4 (1.6)
Baseline eGFR (ml/min per 1.73 m ²), mean (SD)	36.6 (12.0)	36.5 (12.2)	32.7 (11.9)
eGFR (ml/min per 1.73 m ²), mean (SD) ^a	—	−9.0 (12.8)	−19.2 (9.8)
UPCR (mg/g), median (IQR)	0.3 (0.1–1.4)	0.3 (0.1–1.3)	1.2 (0.4–3.3)
hsCRP (mg/L), median (IQR)	2.7 (1.1–6.7)	2.6 (1.1–5.9)	2.5 (1.0–5.9)

^aeGFR: difference between baseline and last eGFR.

Institute Inc, Cary, NC). All *P* values were two-tailed, with an α level of <0.05.

RESULTS

From the 1315 eligible participants in the full CRIC cohort, we studied 894 individuals. Of these 894 individuals, 596 were randomly selected as a subcohort of all eligible CRIC participants (240 of whom subsequently developed DKD progression and thus became cases), and 298 were CRIC participants outside of the subcohort who developed DKD progression and thus became cases during follow-up. Baseline characteristics of the study participants are described in Table 1. The mean age was 60.8 years in the subcohort, and 58.6 years among all cases. A similar frequency of hypertension and current smoking was observed in the subcohort and cases. The mean baseline eGFR was 36.5 ml/min per 1.73 m² in the subcohort and 32.7 ml/min per 1.73 m² among cases, with a median baseline UPCR of 0.3 and 1.2 mg/g among the subcohort and all cases, respectively.

Plasma biomarker concentrations were consistently higher among those with lower baseline eGFR values (Figure 1, Supplemental Figure 2, Supplemental Table 2). Partial Pearson

pairwise correlations between biomarker concentrations and key predictors of CKD progression (eGFR and UPCR) are shown Table 2. TNFR-1 and TNFR-2 were most correlated ($\rho=0.82$), and the pairwise correlations between MCP-1, suPAR, TNFR-1, TNFR-2 were moderate to high ($\rho=0.28$ –0.74). All biomarkers had a consistent inverse correlation with eGFR ($\rho=-0.22$ to -0.73), with the TNFRs and suPAR most strongly correlated. UPCR was positively correlated with all biomarkers ($\rho=0.07$ –0.61).

Association of Plasma Biomarkers with DKD Progression

During the median follow-up of 8.67 years (IQR, 6.44–9.31), there were 538 total events of DKD progression, which included 317 ESKD events and 358 instances of a $\geq 40\%$ decline from the baseline eGFR. When modeling per increment in log₂ biomarker values, higher concentrations of each biomarker was associated with a greater risk of DKD progression, even after adjustment for age, sex, race, education, BP, hsCRP, BMI, smoking, eGFR, and UPCR (Figure 2, model 3 in Supplemental Table 3). There was evidence of an interaction between the baseline eGFR (<45 versus ≥ 45 ml/min per 1.73 m²) and MCP-1 on its association with DKD progression (*P* interaction=0.01), with higher MCP-1 concentrations

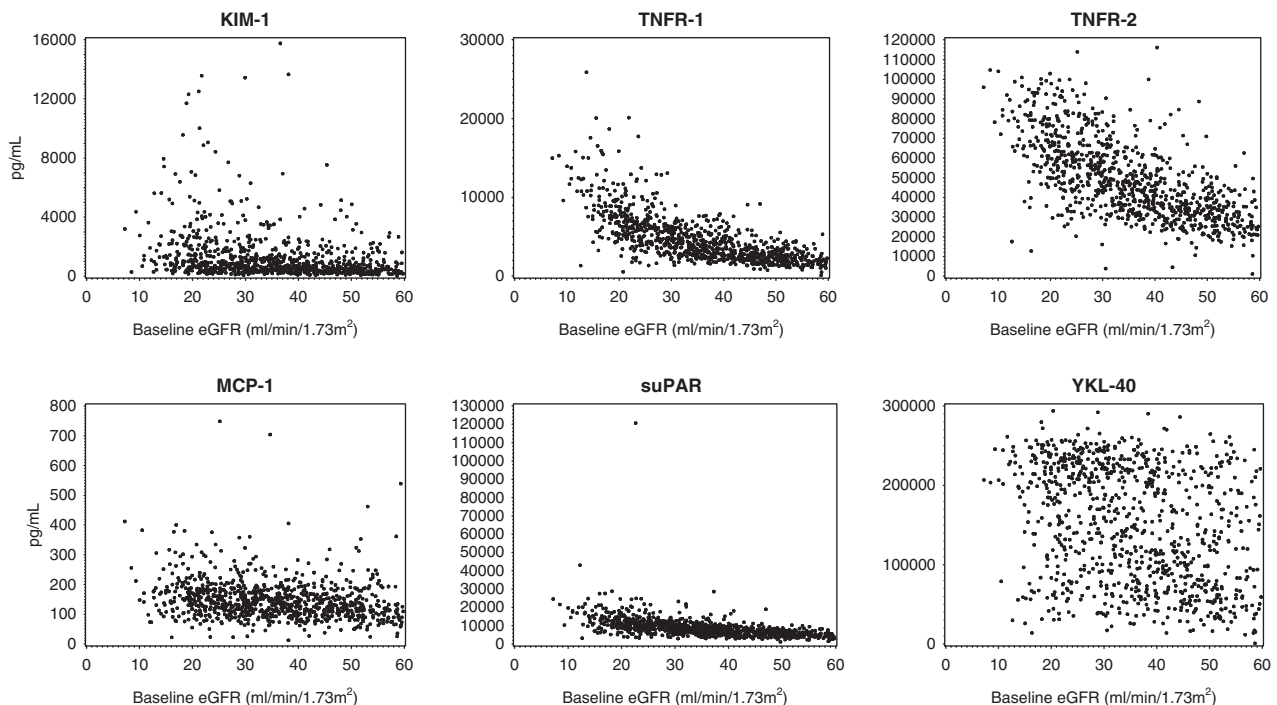


Figure 1. Scatterplot of plasma biomarker concentrations (pg/ml) on y axis were consistently higher among those with lower baseline eGFR (ml/min per 1.73 m²) values on x axis. Top row (left to right): KIM-1, TNFR-1, and TNFR-2. Bottom row (left to right): MCP-1, suPAR, and YKL-40.

associated with increased DKD progression observed for those with eGFR <45 ml/min per 1.73 m², compared with those with eGFR ≥45 ml/min per 1.73 m² (HR, 1.35; 95% CI, 1.16 to 1.56, versus HR, 1.03; 95% CI, 0.81 to 1.31).

The results of analyses using the ComBat approach to address potential batch effects and limiting the Cox regression models to the subcohort were consistent with the results of the primary analysis using weighted Cox regression (Supplemental Tables 4 and 5). Identification of a panel of markers using Cox regression and backward selection identified three markers, KIM-1, TNFR-2, and YKL-40, that jointly remained associated with an increased risk of DKD progression (KIM-1, aHR, 1.17; 95% CI, 1.05 to 1.30; TNFR-2, aHR, 1.61; 95% CI, 1.15 to 2.26; MCP-1, aHR, 1.20; 95% CI, 0.97 to 1.47; YKL-40, aHR, 1.18; 95% CI, 1.01 to 1.39) (Figure 3, Supplemental Table 6).

Association of Plasma Biomarkers with Annual Change in eGFR

After adjustment for age, sex, race, education, BP, hsCRP, BMI, smoking, eGFR, and UPCR; TNFR-1, TNFR-2, KIM-1, and YKL-40 were significantly associated with the annual rate of decline in eGFR (per change in eGFR slope, in milliliters per minute per 1.73 m², per log₂-biomarker per year) (Figure 4, model 3 in Supplemental Table 7).

DISCUSSION

In a cohort of individuals with diabetes mellitus and mild to moderate CKD, we observed that plasma TNFR-1, TNFR-2, KIM-1, MCP-1, suPAR, and YKL-40 concentrations were each associated with DKD progression, even after adjustment for

Table 2. Pearson partial correlations of biomarker levels and eGFR and proteinuria, adjusting for sex and age

Biomarker	MCP-1	TNFR-1	TNFR-2	suPAR	YKL-40	eGFR	UPCR
KIM-1	0.20 ^a	0.48 ^a	0.47 ^a	0.38 ^a	0.31 ^a	−0.34 ^a	0.61 ^a
MCP-1		0.30 ^a	0.32 ^a	0.28 ^a	0.19 ^a	−0.22 ^a	0.07 ^b
TNFR-1			0.82 ^a	0.74 ^a	0.49 ^a	−0.73 ^a	0.44 ^a
TNFR-2				0.71 ^a	0.53 ^a	−0.64 ^a	0.39 ^a
suPAR					0.47 ^a	−0.64 ^a	0.29 ^a
YKL-40						−0.30 ^a	0.27 ^a

All baseline biomarker values are log₂ transformed.

^aP<0.001.

^bP<0.05.

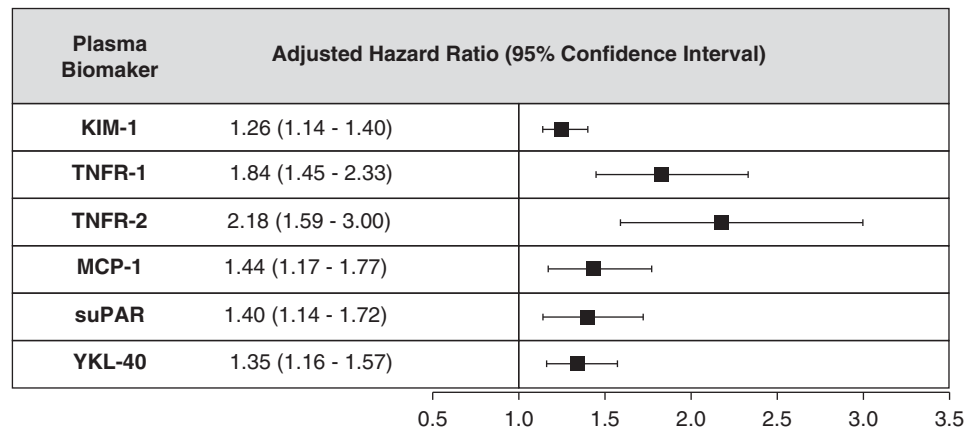


Figure 2. Higher plasma biomarker concentrations (per increment in log2 biomarker values) of KIM-1, TNFR-1, TNFR-2, MCP-1, suPAR, and YKL-40 were associated with a greater risk of DKD progression, even after adjustment for age, sex, race/ethnicity, education, clinical center, systolic BP, diastolic BP, BMI, hsCRP, hemoglobin A1c, antihypertensive medication use, smoking status, baseline eGFR (ml/min per 1.73 m²), and UPCR. All HRs are adjusted for age, sex, race/ethnicity, education, clinical center, systolic BP, diastolic BP, BMI, hsCRP, hemoglobin A1c, antihypertensive medication use, smoking status, baseline eGFR (ml/min per 1.73 m²), and UPCR.

established clinical risk factors; all but MCP-1 and suPAR were also associated with eGFR decline. These findings validate the previous literature on TNFR-1, TNFR-2, and KIM-1, and provide new insights into plasma suPAR and YKL-40 as markers that need validation. Further, higher plasma concentrations of TNFR-2 seem to be most strongly associated with DKD progression after accounting for the other plasma biomarkers.

Worldwide, the number of people with type 2 diabetes mellitus is increasing, and it is estimated that DKD will affect approximately 40% of these individuals,³⁷ and DKD contributes to nearly 50% of new ESKD cases each year.³⁸ The latter observation highlights the importance of identifying and confirming better predictive and prognostic biomarkers for DKD. The pathophysiology of DKD progression is multifactorial and involves inflammation, fibrosis, endothelial dysfunction, tubulointerstitial damage, podocyte injury, extracellular matrix remodeling, and angiogenesis.³⁹ A key pathway noted to be active in DKD progression in humans is inflammation, which is associated with upregulated secretion of several

proinflammatory cytokines, including TNFR proteins (*e.g.*, TNF-R1) and ILs (*e.g.*, IL-1, IL-6, IL-18).^{40,41} At the cellular level, these cytokines have been linked to increasing vascular endothelial cell permeability, glomerular hypercellularity, glomerular basement membrane thickening, inducing apoptosis of endothelial cells, and direct toxicity to kidney cells.^{42–44} Plasma concentrations of TNFR-1 and TNFR-2 are reflective of activation of the TNF pathway. In this investigation, higher concentrations of TNFR-1 and TNFR-2 were associated with DKD progression. These findings are in agreement with prior studies of populations with type 1 or type 2 diabetes mellitus, with normal and decreased baseline eGFR, that demonstrated elevated circulating concentrations of TNFR-1 and TNFR-2 were associated with decline in kidney function (*e.g.*, development of ESKD, macroalbuminuria, or eGFR decline).^{17–21,29} The consistent, strong associations of TNFR-1 and TNFR-2 with DKD progression support their important role in the chronic inflammation of DKD. Only TNFR-2 was associated with DKD progression because

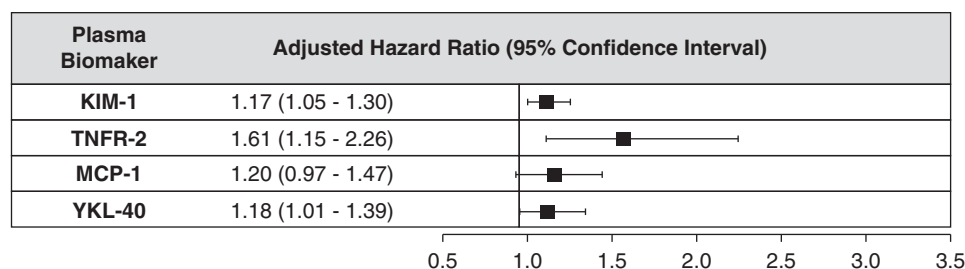


Figure 3. Higher plasma biomarker concentrations (per increment in log2 biomarker values) of KIM-1, TNFR-2, and YKL-40 associated with an increased risk of DKD progression after backward selection of all biomarkers and adjustment for age, sex, race/ethnicity, education, clinical center, systolic BP, diastolic BP, BMI, hsCRP, hemoglobin A1c, antihypertensive medication use, smoking status, baseline eGFR (ml/min per 1.73 m²), and UPCR. All HRs are adjusted for age, sex, race/ethnicity, education, clinical center, systolic BP, diastolic BP, BMI, hsCRP, hemoglobin A1c, antihypertensive medication use, smoking status, baseline eGFR (ml/min per 1.73 m²), baseline UPCR, KIM-1, TNFR-2, MCP-1, and YKL-40. Biomarkers were selected to remain in model via backward selection (*P*<0.05).

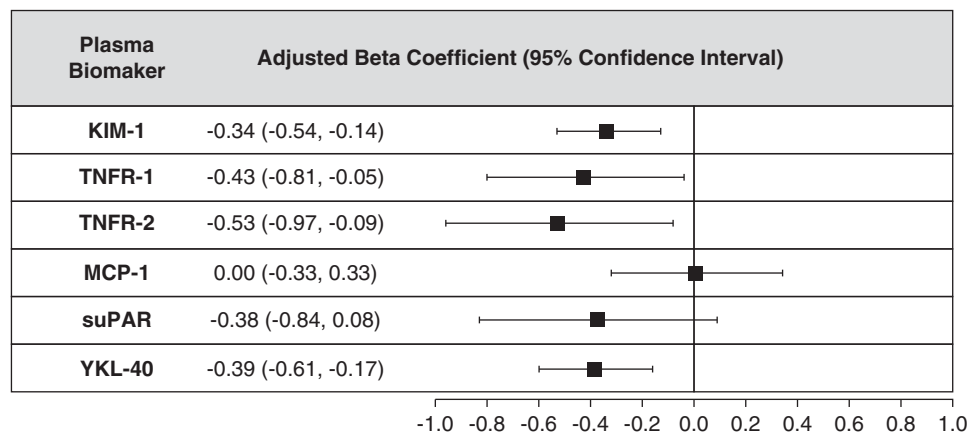


Figure 4. Higher plasma biomarker concentrations (per increment in log2 biomarker values) of TNFR-1, TNFR-2, KIM-1, and YKL-40 were significantly associated with the annual rate of decline in eGFR (ml/min per 1.73 m² per year) after adjustment for age, sex, race/ethnicity, education, clinical center, systolic BP, diastolic BP, BMI, hsCRP, hemoglobin A1c, antihypertensive medication use, smoking status, baseline eGFR (ml/min per 1.73 m²), and UPCR. All β -coefficients are adjusted for age, sex, race/ethnicity, education, clinical center, systolic BP, diastolic BP, BMI, hsCRP, hemoglobin A1c, antihypertensive medication use, smoking status, baseline eGFR (ml/min per 1.73 m²), and UPCR.

TNFR-1 was not retained in the exploratory model that considered all markers jointly, likely because the two biomarkers overlap to such an extent that only one could fit in the model. This finding supports the key role of inflammation in DKD progression.

MCP-1 (or C-C chemokine ligand 2), another inflammatory biomarker, is a member of the C-C chemokine family that leads to the recruitment of monocytes and transformation to macrophages. To date, studies investigating MCP-1 and kidney disease in humans have mostly focused on urine, rather than plasma or serum, MCP-1 concentrations. Urinary MCP-1 has been associated with allograft failure, cardiovascular disease, and death in recipients of kidney transplants^{45,46}; it is also associated with kidney function decline, in individuals with type 2 diabetes who have preserved kidney function,⁴⁷ and macroalbuminuria.²³ However, serum MCP-1 concentrations have not been associated with eGFR decline among those with type 2 diabetes and relatively preserved kidney function.²² This study observed an increased risk of DKD progression with higher plasma MCP-1 concentrations, but only among those with eGFR <45 ml/min per 1.73 m² at baseline, making this the first study to report an association of plasma MCP-1 concentrations and DKD progression among individuals with moderate to severe CKD. Due to the limited investigation of the relationship of blood concentrations of MCP-1 with kidney disease, more research in other study populations with diabetes is warranted to further investigate its role in DKD.

Circulating suPAR, the soluble form of the urokinase-type plasminogen activator receptor which is expressed mainly on immune and endothelial cells, is released into the circulation during inflammation.^{48,49} High suPAR levels have been suggested to activate podocytes in pathologic conditions.⁵⁰ Higher suPAR concentrations were significantly associated

with a larger annual decline in eGFR and an increased risk of incident CKD, defined by eGFR <60 ml/min per 1.73 m², in a study population largely without diabetes (68%).²⁴ In a prospective study of 667 individuals with type 1 diabetes and various degrees of albuminuria, a higher level of suPAR was an independent risk factor for cardiovascular events, $\geq 30\%$ eGFR decline, and mortality.⁵¹ More recently, suPAR was found to correlate with incident ESKD in a Chinese CKD cohort, of which 20% were diabetic.⁵² Consistent with these reports, in this investigation, higher plasma levels of suPAR were associated with an increased risk of DKD progression, even after adjustment for baseline eGFR and albuminuria. In contrast to prior studies, suPAR was not associated with rate of eGFR decline or DKD progression after accounting for competing biomarkers. Importantly, this is the first time that suPAR was measured in the same study cohort as TNFR-1 and TNFR-2. Distinctions between our study and others that examined an association between suPAR and eGFR decline include different assays and mean baseline eGFR (35 ml/min per 1.73 m² in our study versus 50–82 ml/min per 1.73 m²).^{24,51,52} The lack of a correlation between suPAR and the rate of eGFR decline was also noted by Hayek *et al.*²⁴ in the subpopulation with baseline eGFR <60 ml/min per 1.73 m², suggesting the suPAR association may be less prominent or absent in patients with prevalent CKD.

Recurrent or sustained tubular injury is hypothesized to contribute to DKD progression. KIM-1, a marker of acute and chronic proximal tubular injury in both animal models and humans, is detectable in blood and urine after shedding of the KIM-1 ectodomain, consistent with this hypothesis.²⁶ However, previous data on circulating KIM-1 as a biomarker for kidney disease progression has been inconsistent. Serum KIM-1 has been observed to increase across CKD stages and to be associated with eGFR decline and progression to ESKD in

some,^{26,29,53,54} but not all,^{55,56} studies. Prior studies have been limited by small sample sizes and by including those with all levels of eGFR, and individuals without diabetes. In this investigation, higher plasma KIM-1 concentrations were associated with DKD progression and yearly decline in eGFR, which further elucidates the relationship of circulating KIM-1 and kidney function over time. Additionally, even when adjusting for other biomarkers, KIM-1 remained associated with DKD progression. Further research should be conducted to validate the association of KIM-1 with DKD progression.

YKL-40 is an indicator of tubular injury severity and may play a role in limiting tubular cell apoptosis during the repair phase of AKI.³⁰ To date, YKL-40 has mainly been studied as a kidney disease biomarker in the urine and has not yet been extensively studied in blood samples, or in populations with diabetes. Elevated urine levels of YKL-40 have been associated with need for dialysis in kidney transplantation,³⁰ but urinary YKL-40 levels were not associated with eGFR decline in a population with type 2 diabetes.⁴⁷ Blood concentrations of YKL-40 have not been shown to be associated with kidney function changes over time. In one study of participants with type 2 diabetes and varying levels of baseline eGFR (mean eGFR 78 ml/min per 1.73 m²) and albuminuria, plasma YKL-40 was not associated with eGFR decline.²² In this investigation, elevated plasma concentrations of YKL-40 were observed to associate with DKD progression and decline in eGFR over time, even after adjustment for potential confounders and other plasma biomarkers. The panel of biomarkers that was significantly associated with DKD progression, which included inflammatory (TNFR-2) and tubular injury (KIM-1 and YKL-40) molecules, reflects the complex pathobiology of DKD. Further, although the biomarkers had mostly moderate to high intercorrelations, each biomarker alone was associated with DKD progression, suggesting different loci of injury or pathways may be involved in determining outcomes. Ultimately, a multibiomarker approach to risk prediction of DKD progression may be necessary.

Rigorous technical and clinical validation studies are necessary before novel biomarkers can enter clinical practice. Several of the biomarkers examined in this study have previously been shown to be associated with kidney disease progression, although the studies were often small and included heterogeneous etiologies of CKD. To date, many phase-3 clinical trials for DKD have failed,⁵⁷ which may relate to insufficient power, due to low overall rates of progression to end points (e.g., doubling of serum creatinine, onset of ESKD, or death).⁵⁸ Biomarkers may be used in the design of clinical trials to identify patients who are at high risk for developing DKD progression and targeting them for enrollment. Furthermore, novel biomarkers may provide additional, clinically meaningful outcomes, particularly in phase-2 trials. The use of rigorously validated biomarkers for prognostic enrichment of trial populations would reduce trial costs, size, and duration.⁵⁹ Such trials may also be more attractive to potential participants who

are identified by virtue of their high risk of disease progression. We assessed the potential utility of the plasma biomarkers assessed in this study to enrich clinical trial enrollment using the open-source software, Biomarker Prognostic Enrichment Tool for Survival Outcomes (<http://prognosticenrichment.com/surv/>), which is similar to the previously published BioPET for binary outcomes⁶⁰ (Supplemental Material, Supplemental Figures 3 and 4, Supplemental Table 8). As an illustrative example, plasma TNFR-2 could be used to enrich enrollment by excluding individuals at varying concentrations of plasma TNFR-2, such as below the 75th percentile. Assuming no interaction between baseline level of TNFR-2 and treatment effect, it is estimated that the sample size needed to detect a 20% reduction in DKD progression over a 5-year period could be reduced by nearly 50%, with a nearly similar percentage reduction in cost. A similar effect, but to a lesser magnitude, was estimated for KIM-1 (Supplemental Figures 3 and 4, Supplemental Table 8).

The results of this investigation need to be interpreted in the context of some limitations and study strengths. First, due to the observational nature of the study, residual confounding by eGFR is possible because correlations of the biomarkers with eGFR were strong. To address confounding, we leveraged the existence of a well-characterized cohort by adjusting for an extensive set of clinical covariates. Second, participants with elevated biomarker levels may have been recruited into the CRIC Study later in their disease, highlighting the importance of assessment of earlier DKD to avoid index-event bias, which is not fully addressed with adjustment for the baseline eGFR. Third, misclassification is a concern because we did not follow participants after they reached the primary outcome, which would preclude an assessment of an improvement in eGFR. However, 40% eGFR decline represents a large change, which reliably predicts ESKD⁶¹ and conveys the low probability that enough subjects would achieve improved eGFR to substantially influence the results. Fourth, comparison of current and prior results for a specific biomarker may be complicated by ELISA assays using antibodies that recognize different peptide epitopes. Previous studies evaluating biomarkers and DKD have been limited by small sample sizes, especially in the case of participants with type 2 diabetes, and have included participants without kidney disease, which limits the interpretation regarding disease-related pathways. Additionally, all plasma biomarkers were measured in the same laboratory with detailed quality control measures. Additional strengths of this study include the assessment of the relationship of key biomarkers that are likely indicative of pathways important for DKD progression. Sensitivity analyses accounting for batch effects were also consistent with the primary analyses. Furthermore, we assessed these associations in a large, carefully phenotyped, prospective cohort with moderate to severe CKD from clinical centers across the United States that included representation of diverse races and both sexes. These features add to the generalizability of our findings to a typical DKD population.

In summary, higher plasma concentrations of KIM-1, TNFR-1, TNFR-2, MCP-1, suPAR, and YKL-40 were independently associated with increased risk of DKD progression, even after adjusting for known risk factors. These findings validate the previous literature on TNFR-1, TNFR-2, and KIM-1, and provide new insights on plasma suPAR and YKL-40 as markers that need validation. Further, TNFR-2 seems to be most strongly associated with DKD progression after accounting for the other plasma biomarkers. These biomarkers may help in the development of novel therapies to prevent DKD progression and the selection of individuals for clinical trials that will be needed to test their effectiveness.

DISCLOSURES

A. Anderson consulted for Kyowa Hakko Kirin. J. Bonventre declares ownership interests (stock or stock options, excluding diversified mutual funds) with Goldfinch Bio (cofounder and equity holder) and Medibeacon; being a coinventor on KIM-1 patents assigned to Partners Healthcare and thus receives patent rights and royalty payments for intellectual property; and consultancy with Renalytix. J. Bonventre also holds equity in DX Now, Innoviva, Medibeacon, Rubius, Sensor-Kinesis, Sentien, and Thrasos; has received consulting income from Aldeyra, Biomarin, Cadent, Esperion, Praxis, Sarepta, Seattle Genetics, and Sumitomo Dainippon; and has received grant funding from the National Institutes of Health (NIH)/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). J. Bonventre's interests were reviewed and are managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies. S. Coca declares providing consultancy for Bayer, CHF Solutions, Relypsa, RenalytixAI, and Takeda; receiving personal fees from Boehringer-Ingelheim, inRegan, and Quark; and being on the advisory committee of and having ownership interests (stock or stock options, excluding diversified mutual funds) in RenalytixAI. S. Coca also has the patent "Derivation and Validation of a Machine Learning Risk Score using Biomarker and Electronic Patient Data To Predict Rapid Progression of Diabetic Kidney Disease" licensed to RenalytixAI. H. Feldman reports being a scientific advisor or having membership of the steering committee of the CRIC Study; consultancy agreements with DLA Piper, LLP; InMed, Inc.; Kyowa Hakko Kirin Co., Ltd. (ongoing); and National Kidney Foundation; being editor-in-chief of the *American Journal of Kidney Diseases* (member of advisory board); receiving research funding from Regeneron; and receiving honoraria from Rogosin Institute (as invited speaker). O. Gutierrez has received grant funding and consulting fees from Akebia and Amgen, grant funding from GlaxoSmithKline, and personal fees from QED. J. Ix is the principal investigator of an investigator-initiated research grant supported by Baxter International. P. Kimmel reports being an editor of *Chronic Renal Disease*, and being a scientific advisor to or a member of the board of directors of Washington Academy of Medicine. C. Parikh declares having a consulting arrangement with Genfit and Tricida, and having ownership interests (stock or stock options, excluding diversified mutual funds) in and consultancy with Renalytix. V. Sabbiseti declares KIM-1 patents assigned to Partners Healthcare (patent rights). M. Sarnak declares being on the steering committee for Akebia, being on the advisory board for Bayer, and being a consultant for Cardurian. M. Shlipak has served as a consultant for Cricket Health and Intercept Pharmaceuticals, and is a scientific advisor for TAI Diagnostics. S. Waikar reports receiving grants and personal fees from Allena Pharmaceuticals; and personal fees from Barron and Budd (versus Fresenius), Bunch and James, Cerus, CVS, GE Healthcare, GlaxoSmithKline, Harvard Clinical Research Institute (also known as Baim), Johnson & Johnson, Kantum Pharma, Mallinckrodt, Mass Medical International, Pfizer, Public Health Advocacy Institute, Roth Capital Partners, Strataca, Takeda, Venbio,

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SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at <http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2020040487/-/DCSupplemental>.

Supplemental Table 1. Quality control parameters of biospecimen assays.
Supplemental Table 2. Plasma values of biomarkers according to baseline eGFR category ($n=894$).

Supplemental Table 3. Association of plasma biomarkers with risk of DKD progression in staged weighted Cox proportional hazards regression models.

Supplemental Table 4. Association of plasma biomarkers with risk of DKD progression using ComBat approach.

Supplemental Table 5. Association of plasma biomarkers with risk of DKD progression in proportional hazards regression model in the subcohort only.

Supplemental Table 6. Association of plasma biomarkers with risk of DKD progression in proportional hazards regression model adjusting for biomarkers.

Supplemental Table 7. Association of plasma biomarkers with annual change in eGFR (ml/min per 1.73 m^2) in staged linear mixed effects models.

Supplemental Table 8. BioPETsurv analysis of modest to highly prognostic biomarkers for a 5-year clinical trial of DKD progression.

Supplemental Figure 1. Flow diagram of sample selection.

Supplemental Figure 2. Scatterplot of plasma biomarkers (\log_2 -transformed) on y-axis by baseline eGFR (ml/min per 1.73 m^2) on x-axis.

Supplemental Figure 3. BioPETsurv analysis of KIM-1 as a modest prognostic biomarker for a 5-year clinical trial of DKD progression.

Supplemental Figure 4. BioPETsurv analysis of TNFR-2 as a highly prognostic biomarker for a 5-year clinical trial of DKD progression.

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Supplemental Data

- Table S1:** Quality control parameters of biospecimen assays.
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- Table S8:** BioPETsurv analysis of modest to highly prognostic biomarkers for a 5-year clinical trial of DKD progression.
- Figure S1:** Flow Diagram of Sample Selection
- Figure S2:** Scatterplot of plasma biomarkers (log₂-transformed) on y-axis by baseline eGFR (ml/min/1.73m²) on x-axis. Top row (R-L): KIM-1, TNFR-1, TNFR-2. Bottom row (R-L): MCP-1, suPAR, YKL-40.
- Figure S3:** BioPETsurv analysis of KIM-1 as a modest prognostic biomarker for a 5-year clinical trial of DKD progression.
- Figure S4:** BioPETsurv analysis of TNFR-2 as a highly prognostic biomarker for a 5-year clinical trial of DKD progression.

Table S1: Quality control parameters of biospecimen assays.

Assay	Intra-assay*		Inter-assay^		Lower Limit of Detection (pg/ml)	Upper Limit of Detection (pg/ml)
	Mean CV, %	CV% Range	Mean CV, %	CV% Range		
KIM-1	4.3	0 – 21.5	10.0	0.1 - 49.6	1.98	20,000
TNFR-1	7.0	0 – 124.6	14.8	0.5 - 74.1	0.67	16,000
TNFR-2	4.1	0 - 41.4	11.0	0.1 - 51.4	0.17	20,000
MCP-1	3.7	0 - 75	11.2	0.9 - 42.6	0.31	3,900
suPAR	5.4	0 – 129.1	15.3	1.9 - 49.8	53	64,000
YKL-40	2.9	0 - 31.1	9.9	0.2 - 52.9	140	192,000

CV – coefficient of variation; KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR-2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor

*CV represents variability between duplicate pairs on assayed on the same day (N=894)

^CV represents variability between blind duplicates assayed on different days (N=45)

Table S2. Plasma values of biomarkers according to baseline eGFR category (n=894)

	eGFR <30 (N=335)	eGFR ≥30 to <45 (N=335)	eGFR 45 to <60 (N=224)	<i>P</i> *
suPAR (pg/mL)	10,949 (8,784 - 13,796)	7,451 (5,811 - 9,420)	5,599 (4,273 - 6,764)	<0.001
TNFR-1 (pg/mL)	6,391 (4,755 - 8,788)	3,654 (2,628 – 4,861)	2,255 (1,724 - 2,957)	<0.001
TNFR-2 (pg/mL)	62,696 (51,083 – 78,210)	43,033 (34,419 – 52,287)	31,223 (25,277 – 37,547)	<0.001
KIM-1 (pg/mL)	983 (533 – 2,176)	659 (381 – 1,188)	457 (268 - 847)	<0.001
MCP-1 (pg/mL)	156 (119 - 188)	135 (108 - 168)	118 (95 – 154)	<0.001
YKL-40 (pg/mL)	199,091 (127,405 – 230,691)	142,633 (87,687 – 215,445)	97,935 (56,109 – 175,842)	<0.001

Data reported as median and interquartile range. eGFR: estimated glomerular filtration rate (mL/min/1.73m²); suPAR: soluble urokinase-type plasminogen activator receptor; TNFR-1: tumor necrosis factor-1; TNFR-2: tumor necrosis factor-2; KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein-1;

*Assessed with ANOVA or Kruskal-Wallis tests as appropriate.

Table S3: Association of plasma biomarkers with risk of DKD progression in staged weighted Cox proportional hazards regression models. HRs (95% CIs) for DKD progression per unit of log₂-transformed plasma biomarker concentration.

Biomarker*	Model 1 (eGFR)	Model 2 (UPCR)	Model 3 (eGFR + UPCR)
KIM-1	1.58 (1.44-1.73)	1.33 (1.19-1.48)	1.26 (1.14-1.40)
TNFR-1	2.23 (1.76-2.82)	2.41 (2.05-2.84)	1.84 (1.45-2.33)
TNFR-2	2.79 (2.06-3.77)	3.23 (2.51-4.16)	2.18 (1.59-3.00)
MCP-1	1.38 (1.12-1.70)	1.54 (1.25-1.90)	1.44 (1.17-1.77)
suPAR	1.55 (1.28-1.87)	1.95 (1.58-2.40)	1.40 (1.14-1.72)
YKL-40	1.47 (1.27-1.70)	1.47 (1.27-1.71)	1.35 (1.16-1.57)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor; eGFR: estimated glomerular filtration rate (mL/min/1.73m²); UPCR: urine-to-protein creatinine ratio

Base covariates for all models: age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status

Model 1: base covariates plus baseline eGFR

Model 2: base covariates plus UPCR

Model 3: base covariates plus baseline eGFR and UPCR

*per log₂-transformed biomarkers

Table S4: Association of plasma biomarkers with risk of DKD progression using ComBat approach. HRs (95% CIs) for DKD progression per unit of log₂-transformed plasma biomarker concentration.

Biomarker*	HR (95% CI) (Corrected for mean shift)	HR (95% CI) (Corrected for mean and variance shifts)	HR (95% CI) (Corrected for mean shift of quality control samples)
KIM-1	1.30 (1.17-1.44)	1.33 (1.20-1.47)	1.28 (1.16-1.41)
TNFR-1	1.91 (1.49-2.45)	1.83 (1.45-2.32)	1.92 (1.51-2.44)
TNFR-2	2.46 (1.78-3.41)	2.38 (1.75-3.24)	2.59 (1.94-3.45)
MCP-1	1.54 (1.25-1.90)	1.59 (1.29-1.96)	1.56 (1.27-1.92)
suPAR	1.51 (1.22-1.88)	1.57 (1.27-1.94)	1.54 (1.25-1.89)
YKL-40	1.36 (1.17-1.59)	1.39 (1.19-1.62)	1.37(1.17-1.60)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor; eGFR: estimated glomerular filtration rate (mL/min/1.73m²); UPCR: urine-to-protein creatinine ratio

Model 1: adjusted for age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status, UPCR, baseline eGFR

*per 1 unit log₂-transformed biomarkers

Table S5: Association of plasma biomarkers with risk of DKD progression in proportional hazards regression model in the subcohort only. HRs (95% CIs) for DKD progression per unit of log₂-transformed plasma biomarker concentration.

Biomarker*	HR (95% CI)
KIM-1	1.28 (1.12-1.47)
TNFR-1	1.46 (1.07-1.98)
TNFR-2	1.68 (1.16-2.43)
MCP-1	1.38 (1.06-1.80)
suPAR	1.43 (1.02-2.01)
YKL-40	1.30 (1.09-1.56)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor; eGFR: estimated glomerular filtration rate (mL/min/1.73m²); UPCR: urine-to-protein creatinine ratio
Model adjusted for age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status, UPCR + baseline eGFR

*per 1 unit log₂-transformed biomarker

Table S6: Association of plasma biomarkers with risk of DKD progression in proportional hazards regression model adjusting for biomarkers. HRs (95% CIs) for DKD progression per unit of log₂-transformed plasma biomarker concentration.

Biomarker*	HR (95% CI)
KIM-1	1.17 (1.05-1.30)
TNFR-2	1.61 (1.15-2.26)
MCP-1	1.20 (0.97-1.47)
YKL-40	1.18 (1.01-1.39)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-2: tumor necrosis factor 2; eGFR: estimated glomerular filtration rate (mL/min/1.73m²); UPCR: urine-to-protein creatinine ratio
Model adjusted for age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status, UPCR + baseline eGFR

*per 1 standard deviation log₂-transformed biomarker

Biomarkers selected to remain in model via backward selection (p<0.05)

Table S7: Association of plasma biomarkers with annual change in eGFR (ml/min/1.73m²) in staged linear mixed effects models. β (95% CIs) for change in annual eGFR slope per unit log₂-transformed plasma biomarker concentration within the subcohort (N=597).

Biomarker*	Model 1 (eGFR)	Model 2 (UPCR)	Model 3 (eGFR + UPCR)
KIM-1	-0.77 (-0.96, -0.57)	-0.33 (-0.51, -0.14)	-0.34 (-0.54, -0.14)
TNFR-1	-0.97 (-1.38, -0.56)	-0.20 (-0.48, 0.08)	-0.43 (-0.81, -0.05)
TNFR-2	-0.98 (-1.46, -0.50)	-0.29 (-0.64, 0.05)	-0.53 (-0.97, -0.09)
MCP-1	-0.09 (-0.46, 0.28)	0.01 (-0.30, 0.33)	0.00 (-0.33, 0.33)
suPAR	-0.59 (-1.09, -0.09)	-0.20 (-0.56, 0.16)	-0.38 (-0.84, 0.08)
YKL-40	-0.63 (-0.87, -0.39)	-0.35 (-0.56, -0.15)	-0.39 (-0.61, -0.17)

KIM-1- kidney injury molecule-1; MCP-1: Monocyte chemotactic protein; TNFR-1: tumor necrosis factor 1; TNFR-2: tumor necrosis factor 2; suPAR: soluble urokinase-type plasminogen activator receptor; eGFR: estimated glomerular filtration rate (mL/min/1.73m²); UPCR: urine-to-protein creatinine ratio

Base covariates for all models: age, sex, race/ethnicity, education, clinical center, systolic blood pressure, diastolic blood pressure, body mass index, hsCRP, hemoglobin A1c, anti-hypertensive medication use, smoking status

Model 1: base covariates plus baseline eGFR

Model 2: base covariates plus UPCR

Model 3: base covariates plus baseline eGFR and UPCR

*per log₂-transformed biomarkers

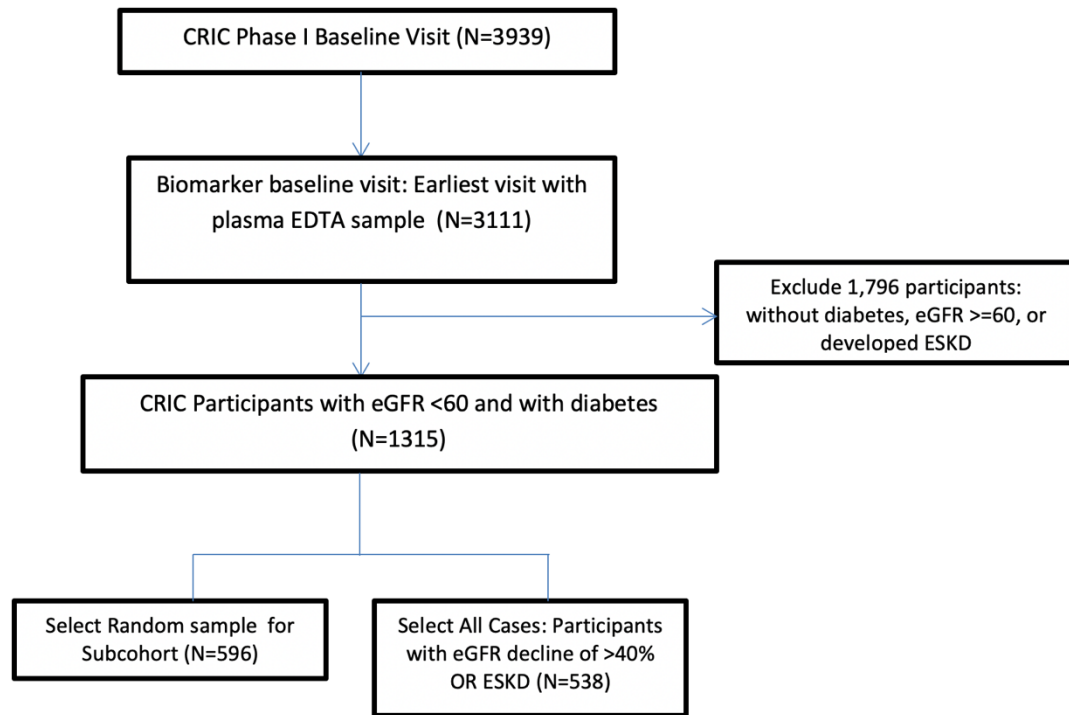


Figure S1: Flow Diagram of Study Sample Selection

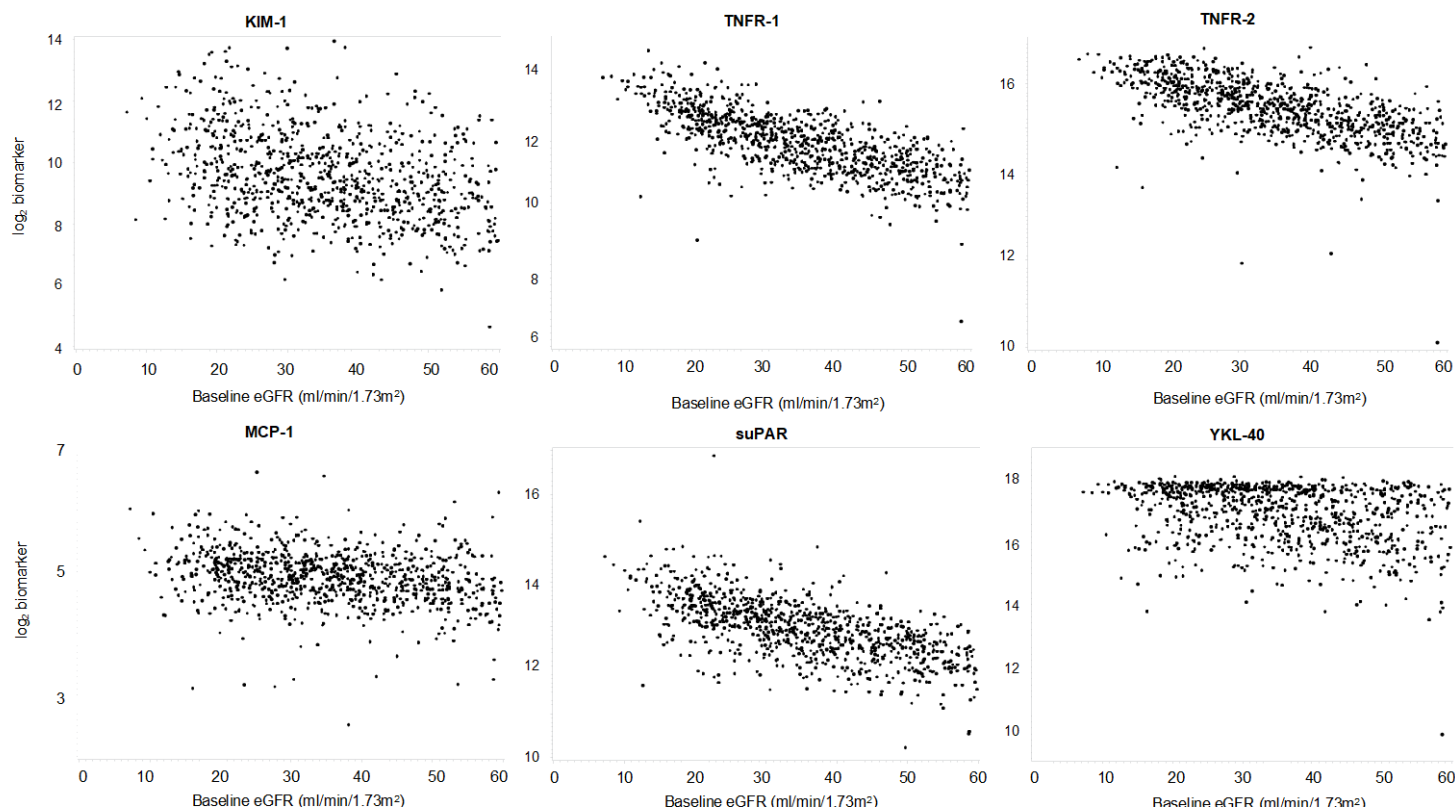


Figure S2: Scatterplot of plasma biomarkers (log₂-transformed in pg/ml) on y-axis by baseline eGFR (ml/min/1.73m²) on x-axis. Top row (R-L): KIM-1, TNFR-1, TNFR-2. Bottom row (R-L): MCP-1, suPAR, YKL-40

Supplemental Methods for Evaluating Plasma Biomarkers for Prognostic Enrichment

The Biomarker Prognostic Enrichment Tool for Survival outcomes (BioPETsurv) is open source software found at: <http://162.243.95.157/surv>, which can assess the potential utility of plasma biomarkers to enrich clinical trial enrollment, and is similar to the previously published BioPET for binary outcomes, located at: <http://162.243.95.157/orig>.¹ BioPETsurv can simulate biomarker and time-to-event data that matches prespecified event rates with and without enrichment in terms of a hazard ratio. BioPETsurv displays Kaplan-Meier survival curves for the entire patient population and enriched subsets. Based on the level of enrichment, the prognostic strength of the biomarker, and length of the trial, BioPETsurv estimates the expected event rate absent intervention. The expected event rate with statistical testing specifications (e.g., power) and the treatment effect determine the trial sample size, which is dependent on the level of enrichment.

We set the BioPETsurv simulation parameters to reflect the range of hazard ratios for DKD progression that were observed to be associated with individual plasma log₂ biomarkers in this study, from the most modest association of KIM-1 (HR 1.26) to the strongest association of TNFR-2 (HR 2.18). We specified constant hazards and normal distribution for the biomarkers. We set the survival data for 5,000 patients with event rate of 25% at 5 years, with 90% power to detect treatment hazard ratio 0.8 (two-sided testing, alpha=0.05), with conservative estimates for cost of screening of \$100 per patient, and cost per-patient of \$100/month to complete the trial.

The simulated results of KIM-1 are shown in **Figure S3**. Panel A shows estimated survival curves for screening threshold 0% (top curve), i.e., for all patients (no enrichment). The plot shows that events accumulate more quickly in enriched subpopulations of patients, showing more quickly decreasing survival curves for enrichment levels 25%, 50%, and 75% (meaning that patients with biomarker below the 25th, 50th, or 75th percentile are excluded). Panel B shows the estimated event rate increases as a function of the level of enrichment. Based on these event rates and specifying 90% power to detect treatment hazard ratio 0.8 (two-sided testing, alpha=0.05), panel C displays the sample size (decreases with greater enrichment). Panel D shows the number of patients needed to screen to enroll for the trial. With higher enrichment, the screening total increases. Panels E and F display the cost analysis, which shows cost

savings for higher levels of enrichment. **Figure S4** displays the simulated results of TNFR-2, the biomarker most strongly associated with DKD progression. **Table S8** displays the numeric results of the simulations.

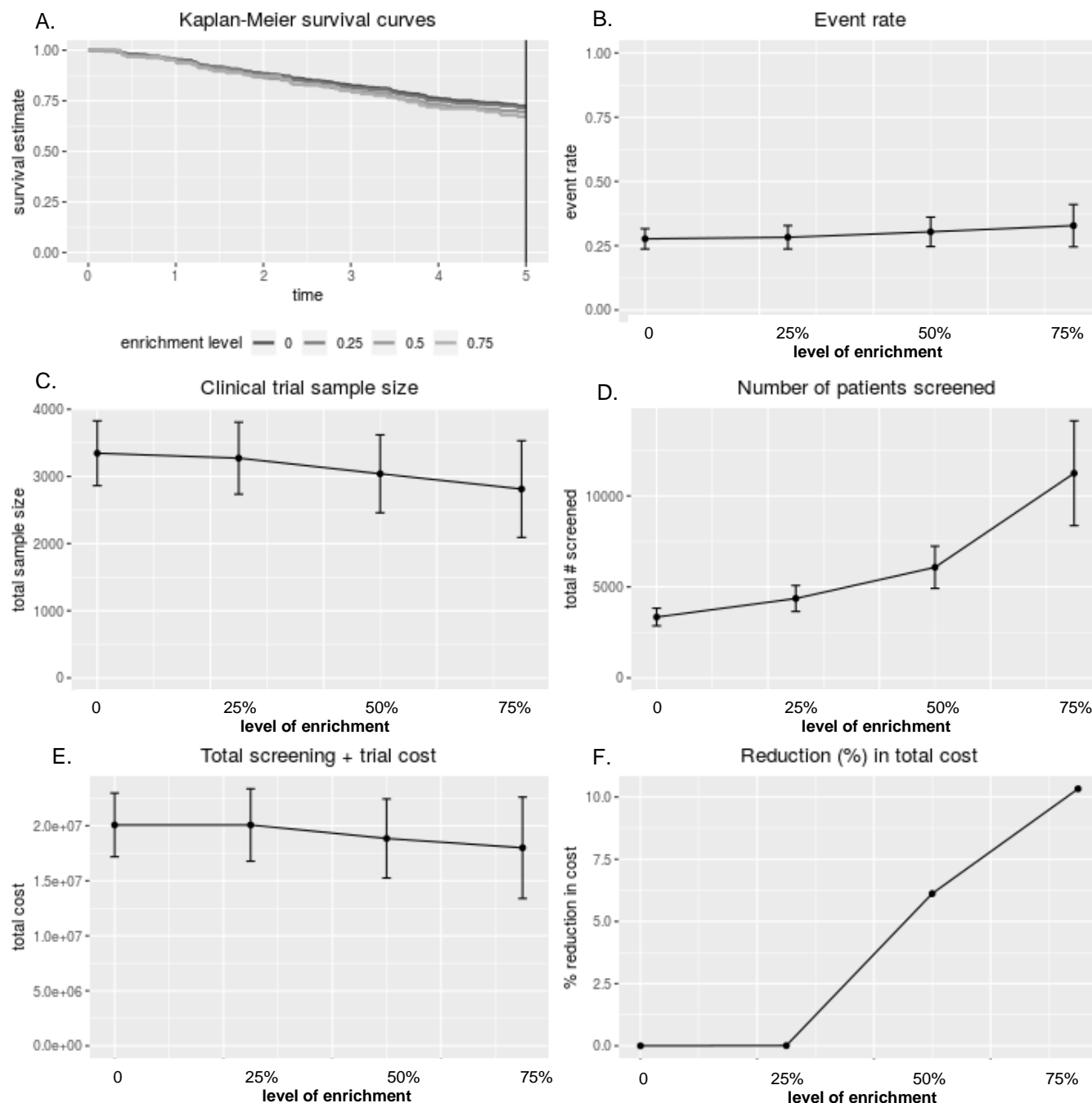


Figure S3: BioPETsurv analysis of KIM-1 as a modest prognostic biomarker of DKD progression for a 5-year clinical trial. The BioPETsurv data simulator generated data for a normally distributed biomarker with prognostic strength of HR 1.26 corresponding to change in \log_2 biomarker. Sample size calculations specified 90% power to detect a treatment hazard of 0.8 using two-sided testing and $\alpha=0.05$. For cost analysis, patient screening was set at \$100 and the cost of a patient in the trial was set at \$100/month.

A.

B.

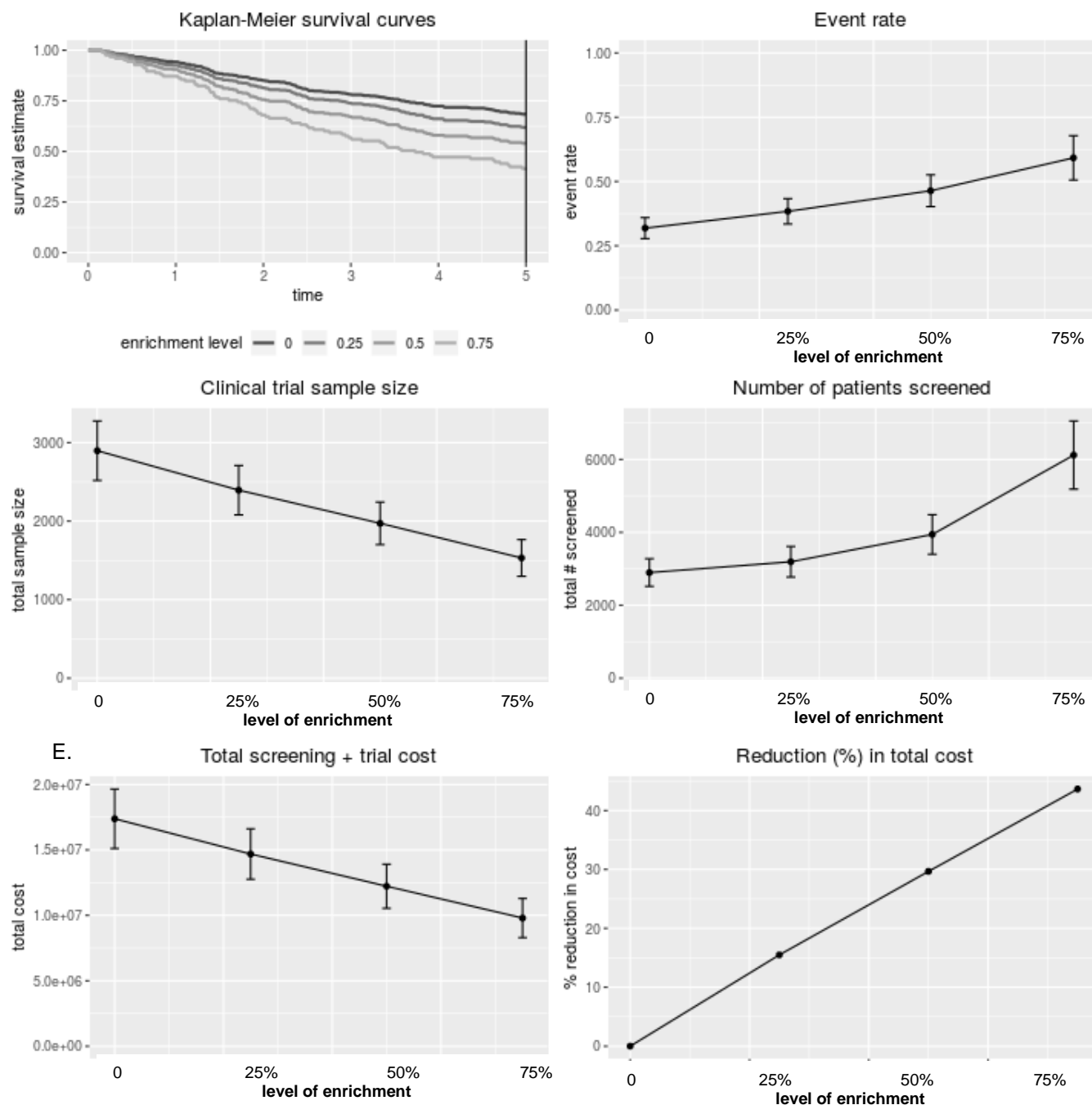


Figure S4: BioPETsurv analysis of TNFR-2 as a highly prognostic biomarker of DKD progression for a 5-year clinical trial. The BioPETsurv data simulator generated data for a normally distributed biomarker with prognostic strength of HR 2.18 corresponding to change in \log_2 biomarker. Sample size calculations specified 90% power to detect a treatment hazard of 0.8 using two-sided testing and $\alpha=0.05$. For cost analysis, patient screening was set at \$100 and the cost of a patient in the trial was set at \$100/month.

Table S8: Simulation of modest to highly prognostic biomarkers of DKD progression for a clinical trial of 5 years.

KIM-1 (HR of 1.26 for DKD Progression)				
Screening Threshold (Level of Enrichment)	Event Rate (%)	Sample Size	Total Screened	Reduction in Total Cost (%)
0%	0.28	3345	3345	0
25%	0.28	3272	4363	0.01%
50%	0.30	3039	6078	6.12%
75%	0.33	2813	11,248	10.3%
TNFR-2 (HR of 2.18 for DKD Progression)				
Screening Threshold (Level of Enrichment)	Event Rate (%)	Sample Size	Total Screened	Reduction in Total Cost (%)
0%	0.32	2896	2896	0%
25%	0.38	2394	3192	15.5%
50%	0.46	1971	3942	29.67%
75%	0.59	1530	6120	43.65%

1. Kerr K, Roth J, Zhu K, et al. Evaluating biomarkers for prognostic enrichment of clinical trials. *Clin Trials*. 2017;14(6):629-638.