Plasma Biomarkers and Kidney Function Decline in Early and Established Diabetic Kidney Disease

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ABSTRACT

Biomarkers of diverse pathophysiologic mechanisms may improve risk stratification for incident or progressive diabetic kidney disease (DKD) in persons with type 2 diabetes. To evaluate such biomarkers, we performed a nested case-control study (n=190 cases of incident DKD and 190 matched controls) and a prospective cohort study (n=1156) using banked baseline plasma samples from participants of randomized, controlled trials of early (ACCORD) and advanced (VA NEPHRON-D) DKD. We assessed the association and discrimination obtained with baseline levels of plasma TNF receptor–1 (TNFR-1), TNFR-2, and kidney injury molecule–1 (KIM-1) for the outcomes of incident DKD (ACCORD) and progressive DKD (VA-NEPHRON-D). At baseline, median concentrations of TNFR-1, TNFR-2, and KIM-1 were roughly two-fold higher in the advanced DKD population (NEPHRON-D) than in the early DKD population (ACCORD). In both cohorts, patients who reached the renal outcome had higher baseline levels than those who did not reach the outcome. Associations between doubling in TNFR-1, TNFR-2, and KIM-1 levels and risk of the renal outcomes were significant for both cohorts. Inclusion of these biomarkers in clinical models increased the area under the curve (SEM) for predicting the renal outcome from 0.68 (0.02) to 0.75 (0.02) in NEPHRON-D. Systematic review of the literature illustrated high consistency in the association between these biomarkers of inflammation and renal outcomes in DKD. In conclusion, TNFR-1, TNFR-2, and KIM-1 independently associated with higher risk of eGFR decline in persons with early or advanced DKD. Moreover, addition of these biomarkers to clinical prognostic models significantly improved discrimination for the renal outcome.

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Diabetic kidney disease (DKD) is a multifactorial syndrome that involves several pathways that result in progressive decline in kidney function. Despite improved understanding of the pathogenesis of DKD over the years, the clinical strategy for prognosticating incident DKD and progression of established DKD still largely depends on traditional markers such as eGFR and albuminuria. However, eGFR and albuminuria are only modestly useful for risk prediction, particularly in patients with preserved levels of renal function.[1] Moreover, several other pathways may be involved with eGFR progression in patients with overt albuminuria, including inflammation, endothelial injury, and tubular injury.[2,3]

One pathway of inflammation that has emerged as central in DKD involves TNF. Gene expression studies have revealed that CKD risk-associated

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transcripts showing an inverse correlation with eGFR clustered around TNF-α.[4] TNF-α directly stimulates podocytes to produce several cytokines, utilizing TNF receptors (TNFRs).[5] TNF cell surface receptors are shed into the extracellular space, including into blood, after cleavage with TNF-α cleaving enzyme. In clinical studies, plasma TNFR-1 and TNFR-2 concentrations have been shown to be associated with the development of ESRD in persons with type 2 diabetes[6,7] and with incident DKD in persons with type 1 diabetes.[8,9]

Kidney injury molecule–1 (KIM-1) is expressed in the apical membrane of proximal tubular cells in response to injury and promotes kidney fibrosis. KIM-1 may enter the circulation because of increased transepithelial permeability or loss of epithelial cell polarity with basolateral membrane expression in early injury. In a small study of persons with type 1 diabetes, plasma KIM-1 levels predicted rate of eGFR loss[10] and ESRD risk during a mean of 10 years of follow-up, after adjustment for confounders.[11]

We hypothesized that plasma TNFR-1, TNFR-2, and KIM-1 would predict eGFR decline in both early and advanced DKD above and beyond clinical variables alone. We measured plasma biomarkers in stored samples from two recently completed clinical trials in patients with early (Action to Control Cardiovascular Risk in Diabetes [ACCORD] trial) and advanced (Veterans Administration NEPHROpathy iN Diabetes study [VA-NEPHRON-D]) DKD and tested their association with eGFR decline.

RESULTS

Participant Characteristics

ACCORD Cohort (Early DKD)

Among the 380 participants (190 matched case-control pairs), the mean age was 62 years. Participants were well matched with regards to age, sex, race, baseline Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) eGFR, and urine albumin creatinine ratio (UACR) (Table 1). Both cases and controls had preserved renal function at baseline (defined as eGFR>60 ml/min per 1.73 m^2). Cases and controls also had similar body mass index (BMI), duration of diabetes, and hemoglobin A1C. In contrast, a greater proportion of cases had a history of cardiovascular disease, cases had slightly higher baseline mean arterial pressure, and a greater proportion of cases were randomized to fibrate intervention. Participants classified as cases had a median of 37 ml/min lower eGFR than controls at the end of 5 years of follow-up (46 versus 83 ml/min per 1.73 m^2; $P<0.01$) but there was no significant difference in UACR. The annual rate of eGFR decline was higher in cases versus controls (7.42 versus 1.67 ml/min per 1.73 m^2; $P<0.01$).

VA-NEPHRON-D Cohort (Advanced DKD)

In NEPHRON-D, those who reached the renal end point were 3 years older, had lower BMI, higher diastolic blood pressure, lower hemoglobin concentrations, and higher LDL concentrations. Median concentrations of the biomarkers were roughly two-fold higher in NEPHRON-D versus ACCORD (Table 1).

Pearson correlations between the log-transformed urinary biomarkers and other DKD risk factors are shown in Table 2. TNFR-1 and TNFR-2 were strongly correlated with each other in both cohorts, and KIM-1 was moderately correlated with TNFR-1 and -2 in both cohorts. The three plasma biomarkers were moderately correlated with UACR in both cohorts, but in NEPHRON-D (advanced DKD), the degree of correlation between the three plasma biomarkers was much stronger than in ACCORD (preserved renal function).

Association of Plasma Biomarkers with Renal End Points in the Two Cohorts

ACCORD Trial Cohort (Early DKD)

Cases had higher baseline levels of TNFR-1 (2526 versus 1963 pg/ml), TNFR-2 (7590 versus 6186 pg/ml), and KIM-1 (254 versus 179 pg/ml) at baseline compared with controls (Table 1). After adjustment for all covariates, including baseline eGFR and UACR, TNFR-1, TNFR-2, and KIM-1 were significantly associated with the renal end point. The adjusted odds ratio (OR) for renal end points per doubling in biomarker was 2.4 (95% confidence interval [95% CI], 1.5 to 4.0), 3.2 (95% CI, 1.7 to 6.1), and 2.4 (95% CI, 1.7 to 3.5) for TNFR-1, TNFR-2, and KIM-1, respectively (model 3 in Figure 1, Table 3). We also stratified the biomarkers by quartiles, and found strong associations for the fourth quartile compared with the lowest quartile: TNFR-1 fourth versus first quartile, adjusted OR, 3.0 (95% CI, 1.2 to 7.3); TNFR-2 adjusted OR, 8.4 (95% CI, 3.0 to 23.4); and KIM-1 adjusted OR, 7.5 (95% CI, 2.8 to 20.0) (model 3 in Supplemental Table 1). There was also evidence of graded signal for the second and third quartiles of TNFR-2 and KIM-1, with no increased risk seen until the third quartile of TNFR-1.

VA-NEPHRON-D Cohort (Advanced DKD)

Those reaching the renal end point had higher baseline levels of TNFR-1 (5481 versus 4095 pg/ml), TNFR-2 (12910 versus 10461 pg/ml), and KIM-1 (735 versus 373 pg/ml) compared with those without the renal end point (Table 1). After adjustment for all covariates, TNFR-1, TNFR-2, and KIM-1 were associated with the renal end point (Table 3). The adjusted OR for renal end points per fold-increase in biomarker was 2.4 (95% CI, 1.7 to 3.3), 2.0 (95% CI, 1.4 to 2.8), and 1.7 (95% CI, 1.5 to 2.1), for TNFR-1, TNFR-2, and KIM-1, respectively (model 3 in Figure 1, Table 3). In quartile analyses with individual markers, TNFR-1 fourth versus first quartile had an adjusted OR, 3.5 (95% CI, 1.9 to 6.3); TNFR-2 adjusted OR, 3.8 (95% CI, 2.0 to 7.3); and KIM-1 adjusted OR, 6.3 (95% CI, 3.3 to 12.3) (Supplemental Table 1).

The rates of eGFR decline by quartiles of each of the biomarkers are shown in Table 4. The rate of eGFR decline was most pronounced for KIM-1, showing a stepwise increase by increasing quartiles. The annual rate of eGFR decline was

Table 1. Clinical and renal marker characteristics in ACCORD nested case-control and NEPHRON-D full sampled cohort

Characteristic	ACCORD			NEPHRON-D		
	Cases (*n*=190)	Controls (*n*=190)	*P*	Renal Outcome (*n*=153)	No Renal Outcome (*n*=1103)	*P*
Clinical characteristics						
Age, yr	62.3 (5.6)	61.9 (5.4)	0.35	62.1 (7.8)	65.2 (7.7)	<0.001
Women	92 (48.4)	93 (48.9)	0.8	1 (0.7)	9 (0.9)	0.76
Race			0.98			0.002
White	141 (74.2)	141 (74.2)		98 (64.1)	764 (76.2)	
Black	20 (10.5)	20 (10.5)		50 (32.7)	203 (20.2)	
Hispanic/Latino	16 (8.4)	16 (8.4)		NA	NA	
Other	13 (6.8)	13 (6.8)		5 (3.3)	36 (3.6)	
Body mass index, kg/m^2	33.4 (5.9)	32.6 (5.5)	0.18	33.61 (6.52)	34.8 (6.8)	0.05
Coronary artery disease (%)	76 (40)	44 (23.5)	<0.01	62 (40.5)	501 (50.0)	0.03
Congestive heart failure (%)	NA	NA		25 (16.3)	160 (16.0)	0.9
Retinopathy (%)	9 (4.9)	26 (13.9)		66 (43.1)	432 (43.1)	0.99
BP, mmHg	97.1 (11.4)	95 (10.3)				
Systolic	135.2 (15.2)	139.8 (18.1)	0.01	137.2 (14.2)	136.2 (16.4)	0.43
Diastolic	74.8 (9·8)	75·5 (10.6)	0.02	73.9 (10.1)	72.2 (10.3)	0.06
Total cholesterol, mmol/L	184.4 (41.7)	174.9 (40.9)	0.04	157 (137–184)	148 (130–179)	0.03
LDL cholesterol, mmol/L	105.5 (33.6)	95.1 (32)	0.01	83 (63–104)	77 (61–97)	0.02
HDL cholesterol, mmol/L	43.9 (11.8)	40.2 (10.4)	0.01	37 (31–43)	36 (31–43)	0.25
Triglycerides, mmol/L	182.1 (104.1)	206.3 (125.6)	0.05	162 (108–227)	165 (112–251)	0.5
Glycated hemoglobin, mmol/mol	8.4 (1.01)	8.4 (1.1)	0.65	7.9 (1.5)	7.8 (1.2)	0.5
eGFR, ml/min/1.73 m^2	87 (77–94)	90 (79–95)	0.31	54 (42–69)	49 (38–65)	0.47
Urine albumin-to-creatinine ratio, mg/mg	21.3 (7.6–66.4)	19.6 (7.7–101.8)	0.45	1739 (714–3211)	781 (465–1597)	<0.001
Medications						
Use of ACEi or ARB at baseline	140 (73.4)	122 (65.2)	0.08	139 (90.3)	917 (91.4)	0.82
Randomized to intensive glycemic control	101 (53.2)	95 (50.8)	0.65	NA	NA	
Randomized to intensive BP control	49 (25.8)	42 (22.5)	0.45	NA	NA	
Randomized to fibrate arm	84 (44.2)	28 (15)	<0.01	NA	NA	
Plasma biomarker concentrations						
TNFR-1, pg/ml	2526 (1872–3308)	1963 (1475–2605)	<0.01	5481 (3579–7067)	4095 (3044–5562)	<0.001
TNFR-2, pg/ml	7590 (6133–9475)	6186 (4924–7787)	<0.01	12910 (9722–16361)	10461 (7998–13388)	<0.001
KIM-1, pg/ml	254 (192·1–226)	179 (137·2–159·2)	<0.01	735 (438–1172)	373 (225–628)	<0.001

Values are presented as mean (SD) for normally distributed continuous values, median (interquartile range) for skewed continuous values, and *N* (%) for categoric values. NA, not applicable or not available. In ACCORD, case is defined as achieving an eGFR<60 mi/min per 1.73 m^2 along with a sustained (on two or more visits ≥3 months apart) decline in eGFR of ≥40% from baseline eGFR. In NEPHRON-D, renal outcome is defined as the occurrence of a decline in the eGFR (an absolute decrease of ≥30 ml/min per 1.73 m^2 if the eGFR was ≥60 ml/min per 1.73 m^2 at randomization or a relative decrease of ≥50% if the eGFR was <60 ml/min per 1.73 m^2) or ESRD (defined by the initiation of maintenance dialysis, receipt of kidney transplant, or an eGFR of <15 ml/min per 1.73 m^2).

5.5 ml/min per year in the fourth quartile versus 2.0 ml/min per year in the first quartile of KIM-1 (P=0.001).

Discrimination and Reclassification in NEPHRON-D

The area under the curve (AUC) for the clinical model alone for the renal end point was 0.68. Addition of TNFR-1 to the clinical model increased the AUC to 0.72 (P=0.003), TNFR-2 to 0.71 (P=0.01), and KIM-1 to 0.74 (P=0.001; Table 5). With all three biomarkers plus the clinical model, the AUC was 0.75 (P<0.001 compared with clinical model alone). These three biomarkers also provided excellent reclassification (net reclassification index) ranging from 0.33 to 0.54 and the integrated discrimination improvement was 0.052 for the clinical factors plus the biomarkers.

Literature Review of Association of TNFR-1 and TNFR-2 with Renal Outcomes

We found four other studies that assessed the independent association between TNFR-1 or TNFR-2 and renal outcomes.[6–8] As shown in the forest plots, both TNFR-1 and TNFR-2 were consistently and robustly associated with the renal outcomes of ESRD, incident CKD, and eGFR decline (Supplemental Figure 1).

DISCUSSION

In two contemporary cohorts of trial participants with type 2 diabetes mellitus (T2DM), we found that plasma TNFR-1, TNFR-2, and KIM-1 were each independently associated

Table 2. Pearson correlations of biomarker levels and baseline participant characteristics

Biomarker	TNFR-2	KIM-1	Age	MAP	eGFR	ACR	HbA1C	BMI
ACCORD								
TNFR-1	0.75[a]	0.28[a]	0.07	−0.02	−0.26[a]	0.25[a]	0.05	0.22[a]
TNFR-2		0.16[a]	0.11[a]	−0.03	−0.38[a]	0.27[a]	0.08	0.28[a]
KIM-1	0.28[a]		−0.02	0.07	−0.05	0.40	0.11[a]	0.01
NEPHRON-D								
TNFR-1	0.81[a]	0.39[a]	0.05	0.06[a]	−0.63[a]	0.34[a]	0.05	0.01
TNFR-2		0.39[a]	0.02	0.05	−0.59[a]	0.35[a]	0.004	0.002
KIM-1	0.39[a]		−0.13[a]	0.14[a]	−0.32[a]	0.49[a]	0.16[a]	−0.09[a]

MAP, mean arterial pressure; UACR, urine albumin creatinine ratio; HbA1C, hemoglobin A1C.
[a]$P<0.05$.

with progressive eGFR decline over time in a strong, graded manner over a broad range of baseline renal function. These associations remained robust even after adjustment for several confounding variables, including baseline eGFR and albuminuria. Most impressive was that the magnitude of association between the continuum of the three markers and the renal outcome was strikingly similar per doubling in concentration within each cohort. This occurred despite the fact that the ACCORD population had virtually no prevalent DKD at baseline, and the NEPHRON-D population had 100% prevalent DKD at baseline. Moreover, the baseline concentrations of these biomarkers were nearly double in NEPHRON-D compared with ACCORD, but the stepwise increase in risk above the risk associated with the lowest values was preserved for both cohorts. Finally, we also found that as compared with a prediction model on the basis of clinical variables, including baseline eGFR and albuminuria, each biomarker demonstrated improved risk prediction for renal outcomes.

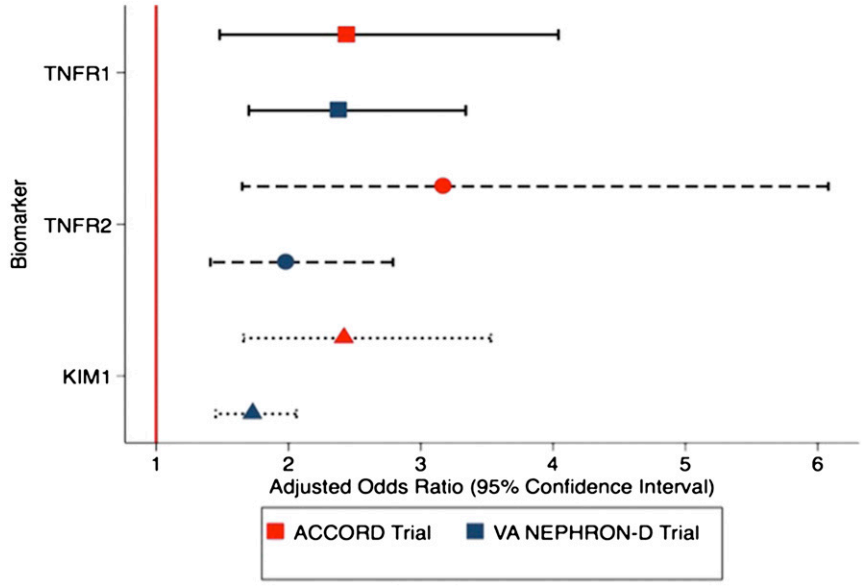

Figure 1. Adjusted OR for the renal outcome per doubling in plasma biomarkers in ACCORD and VA-NEPHRON-D. After adjustment for all covariates, including baseline eGFR and UACR, TNFR1, TNFR2, and KIM-1 were significantly associated with the renal end point. All ORs are adjusted for treatment arm, baseline eGFR, albuminuria, age, race, systolic and diastolic BP, and medications (fibrates/ ACEis/ARBs). In ACCORD, renal end point is defined as achieving an eGFR<60 ml/min per 1.73 m^2 along with a sustained (on two or more visits ≥3 months apart) decline in eGFR of ≥40% from baseline eGFR. In VA-NEPHRON-D, renal outcome is defined as the occurrence of a decline in the eGFR (an absolute decrease of ≥30 ml/min per 1.73 m^2 if the eGFR was ≥60 ml/min per 1.73 m^2 at randomization or a relative decrease of ≥50% if the eGFR was <60 ml/min per 1.73 m^2) or ESRD (defined by the initiation of maintenance dialysis, receipt of kidney transplant, or an eGFR of <15 ml/min per 1.73 m^2).

DKD is a complex, multifactorial syndrome that is the leading cause of ESRD in the United States. Mechanistic understanding into DKD in persons with type 1 and 2 diabetes has revealed that progressive DKD is not only the consequence of hemodynamic and metabolic disturbances, but also due to inflammation. One of the key pathways that are activated in human DKD is the TNF pathway (among others). TNF is a pleotrophic cytokine that is produced predominantly by immune cells that can function in its membrane-bound form or can be released as a soluble circulating 17 kD polypeptide upon cleavage by a metalloproteinase.[12–14] TNF may bind to two transmembrane receptors designated TNFR-1 (p55 or CD120a) or TNFR-2 (p75 or CD120b).[15,16] TNFR-1 is present primarily in glomeruli and endothelial cells whereas TNFR-2 is only expressed transcriptionally in renal cells in various kidney diseases.[17,18] Although TNFR-2 functions as a ligand presenting receptor to TNFR-1, it may have independent functions.[19]

The findings within our study are in accordance with the results from previous studies. Four previous studies have also identified an association between TNFR-1 and TNFR-2 and renal function decline in DKD.[6–9] The results are remarkably consistent across these six cohorts (the 4 previously published plus ACCORD and NEPHRON-D) for the strength of association for both TNFR-1 and -2 with the renal outcomes.

Previous data on plasma KIM-1 as a biomarker for CKD or DKD is not as plentiful as for the TNFRs. KIM-1 is expressed in the apical membrane of proximal tubular cells

Table 3. ORs (95% CIs) for the renal end point[a] per doubling in plasma biomarker concentration in both cohorts

Biomarker	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
ACCORD			
TNFR-1	2.51 (1.68 to 3.77)	2.82 (1.80 to 4.42)	2.44 (1.48 to 4.04)
TNFR-2	4.01 (2.29 to 7.01)	4.22 (2.29 to 7.75)	3.17 (1.65 to 6.08)
KIM-1	1.99 (1.50 to 2.65)	2.00 (1.49 to 2.70)	2.42 (1.66 to 3.53)
VA-NEPHRON-D			
TNFR-1	2.28 (1.73 to 3.03)	2.21 (1.6 to 3.06)	2.38 (1.7 to 3.34)
TNFR-2	2.20 (1.64 to 2.96)	1.97 (1.41 to 2.75)	1.98 (1.41 to 2.79)
KIM-1	1.92 (1.64 to 2.24)	1.83 (1.54 to 2.17)	1.73 (1.45 to 2.06)

All ORs are for a continuous $\log_2$ change in biomarker levels. Model 1: individual biomarker only. Model 2: Model 1 + treatment arm, baseline eGFR, and albuminuria. Model 3: Model 2 + age, race, systolic blood pressure, diastolic blood pressure, and medications (fibrates/ACEis/ARBs).

[a]In ACCORD, renal end point is defined as achieving an eGFR<60 ml/min per 1.73 m^2 along with a sustained (on two or more visits ≥3 months apart) decline in eGFR of ≥40% from baseline eGFR. In VA-NEPHRON-D, renal outcome is defined as the occurrence of a decline in the eGFR (an absolute decrease of ≥30 ml/min per 1.73 m^2 if the eGFR was ≥60 ml/min per 1.73 m^2 at randomization or a relative decrease of ≥50% if the eGFR was <60 ml/min per 1.73 m^2) or ESRD (defined by the initiation of maintenance dialysis, receipt of kidney transplant, or an eGFR of <15 ml/min per 1.73 m^2).

in response to injury and promotes kidney fibrosis. KIM-1 was largely studied in the urine as first a marker for AKI and subsequently as a potential biomarker for CKD. However, there is now knowledge that KIM-1 may enter the circulation because of increased transepithelial permeability or loss of epithelial cell polarity with basolateral membrane expression in chronic kidney injury. In recently published studies in persons with type 1 diabetes, plasma KIM-1 levels were independently associated with eGFR loss in patients with normal renal function[10] at baseline and plasma KIM-1 strongly predicted ESRD risk in patients with type 1 diabetes mellitus and albuminuria.[11]

There are few interventions to prevent DKD or ameliorate progression in those with established DKD. Several agents are being actively tested in clinical trials. However, because of low event rates and long follow-up for event accrual, many trials are inefficient and resource-intensive. Thus, there is a great need for robust prognostic biomarkers for DKD that can allow selective enrollment of those patients with high likelihood of events that would facilitate more efficient clinical trials with higher likelihood of success. In addition, identification of these high-risk participants might be valuable for targeted enrollment in clinical trials, leading to increased events over shorter follow-up periods, ultimately culminating in shorter, efficient trial design. This is consistent with the Food and Drug Administration guidance for enrichment strategies for clinical trials.[20]

Table 4. Annual rate of eGFR decline by quartile of biomarker concentrations in VA-NEPHRON-D

Variable	Mean eGFR Decline in ml/min per 1.73 m^2 per yr (SEM)	*P* Value
TNFR-1, pg/ml		0.01
≤3104	−2.75 (0.18)	
3105–4186	−3.33 (0.17)	
4187–5810	−3.58 (0.18)	
≥5811	−3.33 (0.19)	
TNFR-2, pg/ml		<0.001
≤8182	−2.52 (0.18)	
8183–10699	−3.47 (0.17)	
10,700–13,813	−3.68 (0.18)	
≥13,814	−3.39 (0.20)	
KIM-1, pg/ml		<0.001
≤235	−2.00 (0.17)	
236–406	−3.08 (0.17)	
407–709	−3.34 (0.18)	
≥710	−5.46 (0.21)	

Annual rate of decline derived from linear mixed models accounting for baseline eGFR, treatment arm, biomarker strata, and follow-up time.

Hypothetically, if the panel of the three biomarkers (TNFR-1, TNFR-2, and plasma KIM-1) that has AUC of 0.75 for predicting progression of GFR decline was added as an enrichment criteria for a phase 3 interventional trial of DKD, trial feasibility would be improved through enhanced selection of those patients with DKD most at risk for progression. The sample size reduction with the use of prognostic biomarkers can be simulated under varying assumptions using this website: http://prognosticenrichment.com/.

Some limitations of this study deserve mention. ACCORD and NEPHRON-D were trials conducted before the implementation of SGLT-2 inhibitor drugs, which have been recently shown to decrease risk for progression of kidney disease, as well as cardiovascular disease and death. The VA NEPHRON-D trial was stopped early, at a median of 2.2 years, thus follow-up was not extremely long, and was less than half of the follow-up in ACCORD. Also, there were key differences in the types of participants enrolled into the two trials and the outcomes observed in veterans enrolled into NEPHRON-D may not be generalizable to other populations. However, the consistency of the signals provided by the plasma biomarkers despite the heterogeneity of the case-mix and covariate structure of the two cohorts is also a key strength, and shows the generalizability of the findings across the spectrum of kidney disease.

In summary, TNFR-1, TNFR-2, and plasma KIM-1 levels were associated with higher risk of eGFR decline in T2DM persons with both early (ACCORD) and established (VA-NEPHRON-D) DKD. The consistency of evidence in these two cohorts along with the previously published literature would suggest that the TNFRs have sufficient evidence to be considered for qualification as prognostic biomarkers in T2DM. Moreover, these robust markers can be leveraged as drug development tools to facilitate targeted enrollment of higher risk patients for conducting clinical trials as well as informing better risk prediction in individual patients.

Table 5. Discrimination and reclassification of renal end point by biomarkers in VA-NEPHRON-D

Model	Renal Outcomes					
	AUC	SEM	NRI	SEM	IDI	SEM
Clinical model alone	0.680	0.024	NA	NA	NA	NA
Clinical model plus each biomarker individually						
TNFR-1	0.722[a]	0.022	0.533	0.081	0.024	0.006
TNFR-2	0.709[a]	0.022	0.334	0.086	0.012	0.004
KIM-1	0.735[a]	0.023	0.536	0.083	0.041	0.007
Clinical model plus all biomarkers	0.752[a]	0.021	0.567	0.082	0.052	0.009

Clinical model=treatment arm, baseline eGFR, albuminuria, age, race, systolic blood pressure, diastolic blood pressure, and medications. AUC, area under receiver operative curve; NRI, net reclassification index; IDI, integrated discriminant index; NA, not applicable or not available.
[a]*P* value for Delong test comparing biomarker plus clinical model to clinical model alone was <0.05.

CONCISE METHODS

Participants

The ACCORD Trial

The ACCORD trial enrolled individuals with T2DM, with hemoglobin A1C of 7.5% or more, between the ages of 40 and 79 years with cardiovascular disease, or between the ages of 55 and 79 years with anatomic evidence of significant atherosclerosis, albuminuria, left ventricular hypertrophy, or at least two additional risk factors for cardiovascular disease, across the United States and Canada, and tested three different interventions (glycemic control, BP targets, and fibrates).[21] Individuals with a serum creatinine level >1.5 mg/dl were excluded. Total follow-up of patients averaged over 5 years and kidney function was assessed every 4 months. The study banked blood specimens on participants from the baseline visit and 24 months.

VA-NEPHRON-D

VA-NEPHRON-D was a multicenter, prospective, randomized, placebo-controlled parallel group trial to test the efficacy of the combination of an angiotensin converting enzyme inhibitor (ACEi; lisinopril) with an angiotensin receptor blocker (ARB; losartan), as compared with standard treatment with an ARB alone, on the progression of DKD.[22] Participants were individuals with T2DM with overt albuminuria (>300 mg/g creatinine) and an eGFR between 30 and 89.9 ml/min per 1.73 m^2. The inclusion criteria were designed to select a study sample that was as reflective as possible of the larger population of Veterans Health Administration outpatients with T2DM and nephropathy that would be candidates for ACEi and ARB therapy. The intervention lasted for a median of 2.2 years. Kidney function was assessed every 3 months. The study banked blood specimens on 1256 of the 1448 participants at the baseline visit (Supplemental Figure 2).

Selection of Cases and Controls in ACCORD Trial

In ACCORD, we evaluated the association of the plasma biomarkers with DKD using a nested case-control design, due to the large number of participants in ACCORD and the low event rate. Of the 10,251 ACCORD participants, 3270 had both blood and urine available at baseline and 24 months. We defined incident DKD in ACCORD as achieving an eGFR<60 ml/min per 1.73 m^2 along with a sustained (on two or more visits ≥3 months apart) decline in eGFR of ≥40% from baseline eGFR. This has been shown to be a viable alternative end point for CKD progression and is independently associated with mortality and ESRD.[23] From the 3270, 190 had a sustained eGFR decline of ≥40% and we individually matched them to 190 controls on six key characteristics (age within 5 years, sex, race, baseline mean arterial pressure [within 5 mmHg], baseline albuminuria [within 100 μg/mg], and baseline eGFR within 10 ml/min per 1.73 m^2).

Renal Outcomes in VA-NEPHRON-D Trial

As described in the primary VA-NEPHRON-D trial, the renal end point was the first occurrence of a decline in the eGFR (an absolute decrease of ≥30 ml/min per 1.73 m^2 if the eGFR was ≥60 ml/min per 1.73 m^2 at randomization or a relative decrease of ≥50% if the eGFR was <60 ml/min per 1.73 m^2) or ESRD (defined by the initiation of maintenance dialysis, receipt of kidney transplant, or an eGFR of <15 ml/min per 1.73 m^2). Changes in eGFR were confirmed at least 4 weeks after treatment of potentially reversible factors. All patients with availability of baseline serum and urine samples were included in this study.

Biomarker Measurements

Plasma samples taken at the time of randomization and stored at −80°C in both trial cohorts were used to derive the baseline biomarker measures. Plasma concentrations of TNFR-1 and TNFR-2 were measured *via* the 2-plex 96-well prototype cytokine array from Mesoscale Diagnostics (Meso Scale Discovery, Gaithersburg, MD). The average intra-assay coefficient of variation (CV) was <10% for the calibrators as well as for the quality control (QC) sample. The inter-assay CV for TNFR-1 was 10% and for TNRF-2 was 5.5%–9.28%. The average lower limit of detection obtained from multiple runs was 5.27 pg/ml for TNFR-1 and 0.20 pg/ml for TNFR-2.

Plasma concentrations of KIM-1 were also measured *via* an assay from Mesoscale Diagnostics. The intra-assay CV for the calibrators was <10% and the inter-assay CV was 10.5%, and the average lower limit of detection obtained from multiple runs was 0.5 pg/ml.

Assessment of Covariates in the Cohorts

Seven covariates assessed in the ACCORD trial were used in this analysis. BMI was defined as weight divided by the square of height (kilograms per square meters). BP was on the basis of the average of three measurements using an automated device after 5 minutes' rest with the participant seated in a chair. Serum creatinine was determined using the Roche Creatinine Plus enzymatic assay with spectrometric analysis on a Roche Double Modular P Analytics analyzer (Roche Diagnostics, Indianapolis, IN) and eGFR was calculated from the measured serum creatinine by the CKD-EPI equation.[24] Urine albumin was determined by immunonephelometry on a Siemens BN II nephelometer (Siemens Healthcare Diagnostics, Tarrytown, NY) and urinary creatinine (Cr) by a modified Jaffé reaction. Urinary albumin excretion was estimated from a single spot urine collection by computing the albumin-to-creatinine ratio (Alb/Cr) in units of

milligrams per gram. Medication use, cardiovascular disease, and smoking history were all self-reported by participants. In the NEPHRON-D study, the covariates used in the current analysis were treatment arm, baseline eGFR strata (<60 or ≥ 60 ml/min per 1.73 m^2), albuminuria strata (albumin-to-creatinine ratio ≤ 1000 or >1000 mg/g Cr), age, race, systolic BP, diastolic BP, and other BP medications at NEPHRON-D randomization time.

Statistical Analyses

Primary Analyses

We expressed descriptive results for the participant baseline characteristics and biomarkers as either mean with SD or, in skewed variables, as median with interquartile range. We made statistical comparisons between groups by *t* tests for data that were normally distributed, Wilcoxon tests for skewed continuous data, and chi-squared tests for categoric data.

We evaluated the association of each biomarker (expressed as a continuous log base 2–transformed variable) with the renal end point. In ACCORD, conditional logistic regression was applied for the case-control design. In NEPHRON-D, standard logistic regression was applied where the renal end point occurring any time during the follow-up was counted as an event, and otherwise counted as a nonevent. For each cohort, we considered three models. Model 1 evaluated marginal association by including only individual biomarkers. Model 2 provided partial association by controlling for study design which included treatment arm assignment, baseline eGFR, and albuminuria strata. Model 3 further adjusted for other demographic and clinical variables known to associate with risk for eGFR decline (*e.g.*, age, race, systolic blood pressure, diastolic blood pressure, ACEi/ARB use). We also applied the models 1–3 to the categoric biomarker grouped by quartiles for the renal outcomes.

We used AUC to evaluate biomarker predictive performance for renal end point. We calculated the AUC for the clinical model (*i.e.*, without biomarker) only, clinical model with biomarker each at a time, and the clinical model with all biomarkers. We applied the Delong test to test the significance of improvements in AUC. Finally, we assessed improvement in predictive performance *via* net reclassification index and integrated discrimination improvement index by comparing the clinical model with biomarkers (each at a time and all together) and the clinical model. In NEPHRON-D with the full cohort available, we studied the association of biomarker quartiles with eGFR decline rate using a linear mixed model; using the mixed model, we calculated the annual rate of eGFR decline for each quartile of biomarker.

P values for all end points are two-sided without adjustment of multiple tests; *P* values of <0.05 were considered to indicate statistical significance.

Systematic Review and Meta-Analysis

In order to comprehensively review the existing literature and to construct robust, pooled estimates of TNFRs associated risk for renal outcomes across studies we designed a systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist. We performed a computerized search to identify relevant published studies (January of 2000 to May of 2016). MEDLINE, the Cochrane Library, LILACS, and EMBASE databases were searched using medical subject heading terms and keywords. We included observational cohort studies evaluating TNFR-1 and/or TNFR-2 as predictors of any renal outcome (ESRD, incident CKD 3b, progressive eGFR loss, incident macroalbuminuria). Studies not evaluating renal outcomes were excluded. Two reviewers (G.N.N., S.G.C.) performed independent study selection in duplicate; GNN evaluated and reviewed the selected studies independently and arbitrated disagreements. The final decision regarding inclusion of each study was made by consensus. Forest plots were created; however, we did not provide pooled risk ratios because the units used for the exposure were different in each study. All analyses were conducted using STATA SE, Version 12, College Station, Texas.

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DISCLOSURES

None.

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