REVIEW

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Plasma and urine biomarkers in chronic kidney disease: closer to clinical application

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Purpose of review
Chronic kidney disease (CKD) is a silent disease, causing significant health and economic burden worldwide. It is of strong clinical value to identify novel prognostic, predictive, and pharmacodynamic biomarkers of kidney function, as current available measures have limitations. We reviewed the advances in biomarkers in CKD over the preceding year.

Recent findings
The most frequently studied prognostic plasma biomarkers during recent year were plasma TNFR1, TNFR2, KIM1 and urinary MCP-1 and EGF. New biomarkers such as plasma WFDC2, MMP-7, EFNA4, EPHA2 may also have potential to serve as prognostic biomarkers. There is a shortage of data on biomarkers that are predictive of response to treatments. Data on novel biomarkers to serve as pharmacodynamic biomarkers are limited, but there are emerging data that plasmaTNFR1, TNFR2, KIM-1 are not only prognostic at baseline, but can also contribute to time-updated response signals in response to therapy.

Summary
Data continue to emerge on applicable biomarkers for prognostic clinical risk stratification, prediction of therapeutic response and assessment of early efficacy of interventions. Although more studies are needed for refinement and specific clinical utility, there seems to be sufficient data to support clinical implementation for some biomarkers.

Keywords
biomarkers, chronic kidney disease, prediction, prognosis

INTRODUCTION

It is estimated that 850 million globally have chronic kidney disease (CKD) [1]. In the USA, 37 million have CKD, of which the leading cause is diabetes, which accounts for nearly half of all patients that reach end stage of kidney disease (ESKD). The projected growth for both diabetes and ESKD will impose a significant burden nationally and globally if left unmitigated. However, 90% of patients with CKD have earlier stages that may be treatable before reaching advanced stages that are plagued by irreversible fibrosis and lack any treatment options, other than symptom control. There are now many new treatments for CKD [and diabetic kidney disease (DKD)] that can slow the risk of progression of kidney disease, particularly the sodium-glucose cotransporter-2 inhibitors (SGLT2i). These agents reduce the risk of progression of kidney disease in people with diabetes (DKD) by 40%. However, there are still issues with optimal usage of SGLT2i, including slow implementation [2], potential adverse effects, initial decline in kidney function upon initiation and high costs. The FDA recently approved dapagliflozin to reduce the risk of kidney function decline, kidney failure, cardiovascular death and hospitalization for heart failure in adults with CKD who are at *risk of disease progression.* Although the standard for risk assessment of kidney disease progression is a combination of estimated glomerular filtration rate (eGFR) and proteinuria/albuminuria, the intra-individual variation of

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KEY POINTS

- Available data on prognostic biomarkers for kidney disease progression during last year highlighted the significance of plasma TNFR1, TNFR2, KIM1, WFDC2, MMP-7, EFNA4 and EPHA2, as well as urinary MCP-1 and EGF (each alone and a ratio).
- Plasma biomarkers indicative of treatment response were studied as the posthoc analyses of trials of SGLT2i.
- The most studied pharmacodynamic plasma biomarkers were TNFR1, TNFR2 and KIM-1 during last year.
- More reliable data are needed for further examination of biomarkers and their combination in different biological pathways, particularly for prediction of therapeutic response and pharmacodynamics efficacy.

albuminuria is large, such that decreases of 55% and increases of 124% within 4 weeks are within the bounds of random variation and do not connote true biological change [3]. The same is true for eGFR, where decreases of 16% and increases of 20% can all be due to random variation [3]. Moreover, a substantiation amount of kidney damage (glomerulosclerosis, tubulointerstitial fibrosis) can occur in early stages (particularly in DKD), before there are noticeable decrements in eGFR [4]. Thus, there is a need for more sensitive and specific biomarkers in CKD.

TEXT OF REVIEW

In this comprehensive review, we provide subjective and concise reviews of recent data in the past year on plasma and urine biomarkers in various pathophysiologic domains for prognosis, prediction of response and assessment of pharmacodynamics response in CKD.

Inflammatory biomarkers

Subclinical inflammation is a key process that lurks in the setting of CKD and cardiovascular disease that may be either a manifestation of disease or may serve to worsen ongoing organ damage. Indeed, anti-inflammatory agents have shown efficacy in both reductions of cardiovascular events [5] and kidney disease progression [6]. Through various potential mechanisms, chronic inflammation is associated with the progression of CKD to ESRD.

As the initial seminal work on circulating inflammatory proteins [specifically tumour necrosis factor (TNF) alpha, and TNF receptors (TNFRs)] from the Joslin Diabetes Center in 2012 [7], through their recent Nature Medicine article [8] (expanding on the strength of the entire TNF superfamily as prognostic biomarkers in individuals with diabetes), there have been recent studies that continue to expand upon the totality of evidence of the TNFRs as key biomarkers in CKD (DKD). As part of work conducted by the CKD Biomarker Consortium (CKD Biocon), TNFR1 [adjusted hazard ratio per doubling 1.8, 95% confidence interval (95% CI) 1.5–2.3] and TNFR2 (adjusted HR per doubling 2.2, 95% CI 1.6–3.0) were the two strongest biomarkers for their independent association with progression of prevalent DKD in the Chronic Renal Insufficiency Cohort (CRIC), when compared with other prominent plasma biomarkers (including MCP1, YKL-40, suPAR and KIM-1) [9▪▪]. In the CANagliflozin cardioVascular Assessment Study (CANVAS) cohort, each doubling in baseline TNFR-1 and TNFR-2 were associated with a higher risk of kidney outcomes, with corresponding hazard ratios of 3.7 (95% CI 2.3–6.1; $P < 0.01$) and 2.7 (95% CI 2.0–3.6), respectively [10▪▪]. Additional work from CKD Biocon demonstrated that the TNFRs are also strongly and independently prognostic in settings outside of diabetes. In the CKiD cohort, in children with glomerulonephritits and congenital anomalies of the kidney and urinary tract, plasma TNFR1 and TNFR2 were again the strongest two biomarkers, compared with plasma MCP1, YKL-40, suPAR and KIM-1 [11▪]. Finally, in the African American Study of Kidney Disease and Hypertension (AASK) cohort, each two-fold higher baseline level of sTNFR1 and sTNFR2 was associated with 3.66-fold (95% CI 2.31–5.80), and 2.29-fold (95% CI 1.60–3.29), greater risks of kidney failure [12▪]. These findings remained robustly significant even after adjustment for APOL1 genotype.

Soluble urokinase plasminogen activator receptor (suPAR) is an important regulator of the connection between inflammation, immunity and coagulation. The biomarker is produced by cleavage of membrane-bound suPAR as a result of inflammatory stimuli such as viruses, and cardiovascular risk factors such as smoking and diabetes mellitus. In the aforementioned CRIC and CKiD cohorts, the independent association between suPAR was modest (adjusted hazard ratio 1.4, 95% CI 1.1–1.7 in CRIC [9▪▪]) or not significant (hazard ratio 1.2, 95% CI 0.7–2.2 for quartile 4 vs. quartile 1 in CKiD [11▪]). In a Chinese cohort of 2391 individuals with CKD, plasma suPAR was modestly associated with ESRD (adjusted hazard ratio 1.53, 95% CI 1.10–2.12) [13].

MCP-1 is one of the first chemokines described to play a significant role in renal inflammatory disease. MCP-1 mediates monocytes release from the bone marrow and produces a gradient in the

endothelial glycocalyx directing monocytes to sites of inflammation, thus improving the migration of blood leukocytes into the inflamed tissue [14]. Although plasma MCP-1 did not show significant association with kidney disease progression in the CKiD cohort [11▪], it was modestly associated with progression of DKD in CRIC (hazard ratio 1.44, 95% CI 1.17–1.77) [9▪▪].

Urine MCP-1 was independently associated with eGFR decline among hospitalized patients in the ASSESS-AKI cohort (hazard ratio, 1.32 for each doubling; 95% CI 1.18–1.46) [15], as well as in the SPRINT cohort (adjusted hazard ratio, 2.4; 95% CI 1.1–5.2) [16]. MCP-1 has also been used to index with another biomarker, urinary epidermal growth factor (EGF; discussed further below). In patients with type 2 diabetes in the Joslin Kidney Study, urinary EGF–to–MCP-1 ratio was independently associated with fast kidney function decline, even after accounting for TNFR1, KIM-1 and a novel fibrosis index (MMP7 and WFDC2, which both are discussed later in fibrosis) [17▪▪]. The ratio of postoperative urinary EGF to MCP-1 was weakly associated with future CKD in patients from the TRIBE-AKI cohort [18].

Injury biomarkers

KIM-1 is a marker of tubular injury and a type-1 transmembrane protein expressed in the apical membrane of proximal tubular cells in response to injury. KIM-1 has been shown to be associated with the rate of GFR progression, ESRD, and severity of disease including fibrosis and inflammation, in different stages of CKD [19]. Plasma KIM-1 has also been studied extensively for predicting CKD progression in the past year. In CRIC, plasma KIM-1 was mildly associated with DKD progression (adjusted hazard ratio 1.3, 95% CI 1.1–1.4) [9▪▪]. Similar effect sizes were seen in the CANVAS and Joslin cohorts (adjusted hazard ratio of 1.5, 95% CI 1.2–1.8 and 1.4 (1.1–1.6, respectively). In contrast, plasma KIM-1 was strongly associated with progression in the CKiD cohort (4.5, 95% CI 2.8–8.4 for the fourth vs. the first quartile) [11▪]. Previous studies have demonstrated mixed results on the independent association of KIM-1 with CKD progression. A recent study in the SPRINT cohort demonstrated that urine KIM-1 was strongly associated with CKD outcome (adjusted hazard ratio for the fourth vs. first quartile 2.8, 95% CI 1.3–6.7) [16].

Neutrophil gelatinase-associated lipocalin (NGAL) is an iron-transporting protein that accumulates in kidney tubules and urine after injury. It is an early sensitive biomarker for kidney injury. Urinary NGAL showed association with eGFR decline and kidney transplant in nondiabetic CRIC population (hazard ratio 1.6, 95% CI 1.1–2.3) [20], whereas the association was not significant in those with diabetes [20]. Urine NGAL was not a predictor of CKD progression in the SPRINT substudy [16].

Fibrosis biomarkers

Fibrosis is caused by persistent injury stimuli and leads to organ dysfunction and organ failure. Throughout the pathological process of renal fibrosis, the injured tubular epithelia lose their regenerative capacity and undergo apoptosis. The resulted glomerular scarring progresses to glomerular sclerosis. This leads to tubular atrophy and nonfunctional glomeruli and eventually the progressive kidney disease.

Serum WAP four-disulfide core domain 2 (WFDC2) is a marker of renal fibrosis and has been newly investigated as a clinical prognostic biomarker for kidney disease and fibrosis. Serum matrix metalloproteinase 7 (MMP-7/Matrilysin) has been shown to be involved in the pathogenesis of renal fibrosis. It is a zinc-containing enzyme with proteolytic activity against a wide range of extracellular proteins. High levels of WFDC2 and MMP-7 were associated with kidney function decline and advanced stage of renal fibrosis in type 2 diabetic individuals in the Joslin Kidney Study [17▪▪]. In this population, a combination level of WFDC2 and MMP-7, considered as 'fibrosis index', was strongly associated with renal decline regardless of albuminuria status (odds ratio per doubling 1.63; 95% CI 1.30–2.04) [17▪▪].

YKL-40 is an emerging heparin and chitin-binding glycoprotein indicating structural kidney damage and tubular fibrosis in different clinical settings. Plasma YKL-40 showed weak associations with kidney disease progression in some cohorts (e.g. association with progressive DKD in CRIC study) [9▪▪]. The additive value of plasma YKL-40 to predictive biomarkers of TNFRs and KIM1 for kidney disease progression is still unclear. In children, population of CKiD cohort, plasma YKL-40 was nominally associated with progression (adjusted hazard ratio 1.33, 95% CI 0.83–2.4), but did not significantly improve the predictive performance of the model, including plasma TNFRs, KIM1 and routinely measured clinical variables [11▪]. Urinary YKL-40 weakly associated with eGFR decline and incident composite renal outcome over time in the hospitalized patients of a multicentre cohort (1.15; 95% CI 1.09–1.22) [15].

Other biomarkers

Fibroblast growth factor 23 (FGF-23) is a hormone that regulates phosphorus levels and vitamin D

metabolism. FGF-23 has been shown to be associated with incident dialyses or kidney transplant in CRIC population (hazard ratio 1.18, 95% CI 1.02–1.37) [21]. In PREVEND study, FGF-23 was associated with incident CKD (hazard ratio 1.25, 95% CI 1.10–1.44) [22].

Urinary epidermal growth factor (uEGF) has been a possible biomarker of kidney function, as its receptor play an essential role in cell growth, migration, proliferation and differentiation. Urinary EGF recently showed as a promising biomarker of CKD progression with significant association with rapid eGFR decline among white populations of RENIS (hazard ratio 1.42, 95% CI 1.06–1.91) [23▪] and in population of PREVEND (hazard ratio 1.29, 95% CI 1.10–1.53) [22].

Other biomarkers that have been frequently studied in kidney diseases, such as urinary beta-2 microglobulin (B2M), IL-18 and uromodulin (UMOD), did not show significant association with ESKD or eGFR decline in SPRINT [16,24].

The axon guidance pathway (AGP) is important in the development of the nervous system. AGPs may have important roles in the development and repair of many cell types in vascularized tissues, and in processes including kidney angiogenesis and blood vessel maturation. Circulating AGP proteins strongly correlate with early structural kidney lesions. In the Joslin Kidney Study cohort, these markers independently associated with ESKD in both type 1 and type 2 diabetes [adjusted hazard ratio per quartile increase for Ephrin A4 (EFNA4) and EPH Receptor A2 (EPHA2) were 1.6–2.0 in type 1 diabetes and were 1.7–2.0 in type 2 diabetes] [25▪▪].

Potential uses of biomarkers

Prognostic

Prognostic biomarkers may be useful in the clinical arena to risk stratify patients for intensity of clinical care and referrals, or in the design and conduct of clinical trials by *a priori* defining the population patients who are at a high risk for CKD (DKD) progression and targeting them for enrolment. The use of validated biomarkers to enrich a trial population can be potentially cost-effective by lessening the sample size necessary to detect a statistically significant finding of a given intervention [26].

In a recent analysis of participants in the CRIC cohort, the potential utility of the plasma biomarkers was assessed using an open-source software at prognosticenrichment.com. Plasma TNFR-2 showed the utility to enrich enrolment by excluding individuals at varying concentrations of plasma TNFR-2, such as below the 75th percentile. If no interaction between baseline level of TNFR-2 and treatment effect, the sample size needed to detect a 20% reduction in DKD progression in 5-year period was reduced by nearly 50%, with almost similar percentage decline in costs. Plasma KIM-1 also showed a similar ability for enrichment, albeit to a lesser degree than TNFR2 [26].

KidneyIntelX is a new composite risk score that incorporates three of the aforementioned plasma biomarkers (TNFR1, TNFR2 and KIM-1) with seven clinical variables and creates a composite risk score for progression of kidney disease over 5 years in patients with stages 1–3 DKD. In a validation study, using samples from Mount Sinai Biome and UPenn, the model was tested, trained and validated to produce three levels of risk (low, intermediate and high) as well as a continuous risk score. In patients who scored high risk (16.5% of the population), 61% experienced kidney disease progression (defined by a 5 ml/min/year decline in eGFR, a 40% sustained decline or kidney failure). In those who scored low risk (the bottom 46%), only 10% had progression of kidney disease [27▪▪]. KidneyIntelX is currently CLIA-approved in 50 states and testing in New York has recently initiated, with additional sites regionally and nationally to commence soon. The commercial use includes dedicated care-path recommendations tied to the three levels of risk.

Predictive of response

Prognostic biomarkers identify individuals who are at a higher risk of the outcome, but do not necessarily predict individuals more or less likely to respond to various treatments for kidney disease. Albuminuria is a classic marker that in general, does identify patients more likely to derive beneficial renal response to ACEi/ARBs [28]. NT-pro BNP and other markers have shown predictive abilities for therapies in patients with heart failure [29].

The hope has been that more in-depth phenotyping of patients with serum and urine biomarkers (that are already classified as 'prognostic' or other markers that may have interactions with the proposed mechanism of action of drug) may reveal those that are more likely to be responsive to therapies for CKD or DKD. Recent successful trials with the SGLT2i have provided an opportunity to examine whether some of the biomarkers can 'predict those most likely to respond'. Posthoc analyses of the CANVAS and Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trials have demonstrated that proportional effects of canagliflozin (SGLT2i) on renal are mostly consistent across patients with different levels of albuminuria, but absolute benefits

are greatest among those with severely increased albuminuria [30,31]. Although these posthoc analyses on the banked samples from several large RCTs for biomarkers of interest are still in progress/ongoing (CANVAS, CREDENCE and others), there are some newly available data from CANVAS [10▪▪].

As mentioned in the prognostic section above, plasma TNFR1, TNFR2 and KIM-1 were recently measured in the CANVAS cohort. Although TNFR1 and TNFR2 were prognostic for the kidney outcome, there was no evidence that the effects of canagliflozin on the kidney outcomes varied by the baseline level of the individual plasma biomarkers, TNFR1 and TNFR2 and KIM-1 [10▪▪]. Subsequent analyses examined the three plasma biomarkers (TNFR1, TNFR2, KIM-1) within the framework of the KidneyIntelX bioprognostic test. Using this comprehensive risk score in participants with DKD at baseline ($n = 1325$), the treatment effect of canagliflozin vs. placebo on chronic eGFR slopes differed by KidneyIntelX strata. There was evidence of greater protection, as measured by difference in eGFR slope for canagliflozin vs. placebo, in those with higher KidneyIntelX risk category (placebo-subtracted eGFR slope: 0.66 ml/min/1.73 m^2 in low risk, 1.52 ml/min/1.73 m^2 in intermediate risk and 2.16 ml/min/1.73 m^2 in high risk). The differences in eGFR slope for canagliflozin vs. placebo in the high-risk KidneyIntelX stratum (2.16 ml/min/1.73 m^2) were of greater magnitude when compared with the effect of canagliflozin vs. placebo in the highest KDIGO risk stratum (1.31 ml/min/1.73 m^2; $P < 0.001$) [32].

Pharmacodynamic biomarkers

Biomarkers that reflect early indications of efficacy on kidney tissue or outcomes would be beneficial, particularly for phase 2 trials of novel agents. The time needed to show that therapies slow progression of eGFR decline or ESKD can take many years. Biomarkers of inflammation, fibrosis, kidney injury or other pathways may yield signals that precede the changes in eGFR by many years. This is particularly important for drugs that reduce intraglomerular pressures (e.g. ACEi/ARB, SGLT2i, mineralocorticoid receptor antagonists), as the initial decline in eGFR causes a lag of 12–24 months for eGFR slope to be equal to or improved compared with placebo. In contrast, drugs such as bardoxolone increased eGFR within 4 weeks of therapy compared with placebo (BEAM trial) [33], but did not translate into an improvement of clinical kidney or cardiovascular outcomes (BEACON trial) [34]. A marker that reflected response with 3–6 months would be highly rewarding.

In a clinical trial setting, canagliflozin treatment indeed decreased TNFR1, IL-6, MMP7 and FN1 levels compared with glimepiride treatment, suggesting that canagliflozin treatment contributes to the reversal of molecular processes related to inflammation, extracellular matrix and fibrosis. sTNFR1 was the only biomarker for which the change was significantly associated with decline in eGFR ($P = 0.026$ when modelled as a continuous variable), independent of other risk markers of kidney function decline [35].

In another posthoc analysis of the CANVAS trial population, in which TNFR1, TNFR2 and KIM-1 were measured at years 1, 3 and 6 years after enrolment, canagliflozin attenuated the increase in TNFR-1, TNFR-2 and KIM-1 by 3–27% compared with placebo [10▪▪]. In multivariable analyses, after adjustment for all covariates, each 10% reduction in TNFR-1 and TNFR-2 was independently associated with a 10–20% lower risk of the kidney outcome. However, although SGLT2i decreased KIM-1 compared with placebo, the changes in KIM-1 from baseline to year 1 did not independently associate with kidney outcomes. The association between 1-year changes from baseline in TNFR-1 and TNFR-2 and kidney outcomes were consistent in the placebo

Table 1. Potential use cases for various biomarkers and key references

Potential uses of biomarkers	Purpose of use	Significant examples	References
Prognostic	Risk Stratify for Outcomes	Plasma TNFR1, TNFR2, KIM1, WFDC2, MMP-7, EFNA4, EPHA2	Greenberg *et al.* [11▪] Ihara *et al.* [17▪▪] Chen *et al.* [12▪] Satake *et al.* [25▪▪] Schrauben *et al.* [9▪▪]
Predictive of response	Predict response to therapy (or lack thereof)	TBD	TBD
Pharmacodynamic	Assess response to therapy	Plasma TNFR1, TNFR2, KIM-1	Chen *et al.* [37] Sen *et al.* [10▪▪]

KIM1, Kidney Injury Molecule-1; MMP-7, matrix metalloproteinase-7; TBD, to be defined; TNFR, tumour necrosis factor receptor; WFDC2, WAP Four-Disulfide Core Domain 2.

and canagliflozin groups (p for interaction 0.60 and 0.20, respectively) (Table 1). Similar findings were seen for the KidneyIntelX composite test, in that SGLT2i decreased KidneyIntelX over time, and the changes over time were prognostic of future kidney outcomes [36]. More data demonstrating that the TNFRs can potentially serve in some pharmacodynamic capacity comes from a recent analysis of the VA NEPHRON-D trial. In this cohort with DKD, each doubling in sTNFR1, sTNFR2, and KIM-1 from baseline to 1 year was associated with 2.9 (1.8–4.6), 1.6 (1–2.3) and 1.3 (1.0–1.6) increased risk of subsequent kidney function decline, respectively, and independent of treatment arm and other covariates, including time-updated eGFR and UACR at 12 months [37].

CONCLUSION

The last decade of investigations has led to robust analyses of various blood and urine biomarkers, with data in the last year that suggest that we have now reached the tipping point for uses of some biomarkers or combinations of biomarkers for prognostic risk stratification in clinical settings and clinical trials (enrichment), predict response and monitor early efficacy of interventions. Although additional studies are needed for further refinement as well as assessment of broader combinations of biomarkers from various biological pathways, the totality of evidence suggests some of the biomarkers are ready for implementation. The next wave of data will certainly examine the potential clinical utility of these biomarkers or biomarker scores and how they may improve processes of patient care and ultimately clinical outcomes. A prime example would be to ensure that high-risk patients are treated with the new therapies that have been shown to slow progression (e.g. SGLT2i, GLP-1 agonists, Finerenone). The clinical and scientific community in nephrology has awaited this exciting era for many years. There is no time like to present to maximize the attempts to preserve kidney health.

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Conflicts of interest

There are no conflicts of interest.

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