nature reviews nephrology

https://doi.org/10.1038/s41581-025-01008-2

Review article

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Urine as a source of biomarkers and biological knowledge in chronic kidney disease

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Abstract

Albuminuria and estimates of glomerular filtration rate remain the main diagnostic and monitoring metrics used in people with chronic kidney disease (CKD). Although these are both useful markers of kidney disease, they represent the consequence rather than the cause of CKD, can neither detect disease at its earliest stages nor determine its aetiology, and are often suboptimal in guiding therapeutic intervention. By contrast, nucleotide, protein, peptide and metabolite findings from urine can provide a wealth of information about kidney-tissue biology and pathological processes, thereby representing a source of potential biomarkers for early disease detection, prognostication and therapeutic guidance. Urinary biomarker research is currently dominated by studies of protein biomarkers that reflect tissue injury and repair, inflammation and fibrosis, and can be combined for use in multi-marker panels. Data on biomarkers for guiding therapy are scarce, underscoring the urgent need for more targeted studies, given the availability of several new therapies that are effective in attenuating CKD progression and improving patient outcomes. Consequently, although several (mainly protein) biomarkers with evidenced potential to improve disease management are currently available, their clinical implementation is limited by the paucity of clinical and health-economic impact data, especially data on the combined use of urinary biomarkers and the latest therapies available for people with CKD.

Sections

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Key points

- Urine analysis is equivalent to a liquid biopsy that can provide insights into kidney pathophysiology. Urinary cellular and molecular components, analysed using contemporary techniques, provide rich biological and clinical information.
- Biologically relevant changes in urinary mitochondrial and nuclear DNA, and various RNA variants have been linked to chronic kidney disease. However, the follow-up studies needed to define their biomarker value are scarce.
- Metabolomic studies have highlighted uraemic toxins and metabolites of potential biomarker value, including amino acids and lipids. Yet, the vast complexity of the metabolome and confounding factors hamper biomarker validation.
- Protein biomarkers reflecting tissue injury, repair, inflammation and fibrosis have been validated, with potential applications for early and differential diagnosis and prognostication of chronic kidney disease; studies on biomarkers guiding therapy remain scarce.
- The clinical and health-economic impact of applying promising biomarkers in combination with the latest interventional approaches must be evaluated to facilitate their implementation.

Introduction

Chronic kidney disease (CKD) is defined by the presence of kidney damage and/or glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 for ≥3 months, irrespective of cause. In many cases, kidney damage can be ascertained by the presence of albuminuria, defined by an urinary albumin-to-creatinine ratio (UACR) > 30 mg/g in two of three spot urine specimens. Additional signs of kidney damage include urinary sediment changes, structural modifications evident on kidney imaging and electrolyte disturbances caused by tubular dysfunction[1]. Serum creatinine, estimated GFR (eGFR) and UACR are thus valuable tools and are currently the main biomarkers used for CKD detection and prognosis. However, these markers explain intra- and inter-individual variability insufficiently and are late indicators of kidney damage[2,3]. Over 70 equations for GFR estimation have been proposed, taking into consideration patient demographics, and other clinical and pathological characteristics, but they are nonetheless unable to match the accuracy of measured GFR as a reflection of kidney function[4]. Moreover, current markers cannot be used to determine the cause of kidney damage and are suboptimal in guiding therapy[2]. Histopathological analysis of kidney-biopsy tissue remains the most accurate diagnostic tool (albeit with limited prognostic value), therefore representing the 'gold standard' in disease diagnosis and monitoring. However, the invasive nature and associated risks of kidney biopsies limit their use for longitudinal follow-up of people with CKD.

Researchers have proposed numerous plasma and urine biomarkers of CKD that are often independently associated with disease progression, even when adjusted for CKD risk factors, including eGFR and albuminuria[5–8]. Despite a clear need for further refinement, these new biomarkers collectively reflect diverse biological processes, including inflammation, coagulation abnormalities and fibrosis, as well as physiological and functional alterations in the kidneys, such as tubular injury. These findings therefore suggest the potential development of a 'liquid biopsy' to monitor CKD progression and guide patient management non-invasively in the future[6,9,10]. Furthermore, defining biomarker-based surrogate endpoints in clinical trials could reduce trial duration, the number of participants needed and, thus, the associated costs. Biomarkers could also help with patient stratification at clinical-trial enrolment through estimates of disease progression or likelihood of therapeutic response, thus increasing success rates and collectively maximizing resource use[4,5,8].

However, despite considerable progress, clinical implementation of biomarker findings has not yet been achieved. Contributing factors include the complex underlying disease pathophysiology, lack of a clear roadmap for biomarker implementation following discovery, the long duration of clinical trials (inherent to chronic diseases) needed to meet regulatory requirements, the need for vast clinical and financial resources, health-economic restrictions, and the reluctance of clinicians, health insurers and policymakers to change the status quo[5,8].

In this Review, we provide an overview of key molecular findings that can be obtained from urine, with a focus on research conducted over the past 5 years. We discuss several biomarkers, including their ability to reflect kidney-tissue biology and their potential for use in early disease detection, prognostication and for guiding therapeutic intervention. Our discussion is limited to CKD (with the acknowledgement that the definition of CKD of various aetiologies in the studies included here does not strictly conform with the CKD definition discussed earlier[1]) and excludes acute kidney injury (AKI), except where AKI progression to CKD was investigated. Furthermore, paediatric data have not been included, given the frequent distinct pathophysiology of paediatric kidney disease, which is often linked to congenital kidney anomalies rather than systemically induced dysfunction. We have sought to discuss evidence that is representative of current urinary CKD biomarker research, with the acknowledged limitation that not all biomarker findings can be discussed, given the vastness of the field.

Investigating kidney function and metabolism through urine

Urine can be collected non-invasively and provides direct insights into kidney-health status and pathophysiology. The cellular and molecular components of urine, including shedded cells, extracellular vesicles and a plethora of soluble factors representing all molecular levels (nucleotides, proteins, metabolites and ions) (Fig. 1), provide a wealth of biological and clinical information. However, the concentration ranges of these components vary widely as they are influenced by nutritional and hydration status, and body composition. In addition, currently used normalization approaches (mainly dividing urinary concentration by creatinine) are not perfect[11] given that they, themselves, can be affected by changes in kidney function and other clinical and biological variables[12]. Compared with blood, urine is particularly suited to proteomic analysis because of its greater stability[13]. Of note, although analyses of blood, plasma or serum are also affected by wide concentration ranges and have high complexity[14,15], they have the advantage of providing information about systemic changes and have yielded promising biomarkers for CKD (reviewed in Zabetian and Coca[5], Villalvazo et al.[16] and McDonnell et al.[17]). Nonetheless, in kidney diseases, urine might reflect changes in the kidneys better than blood, particularly in the early stages of CKD, irrespective of changes in glomerular filtration.

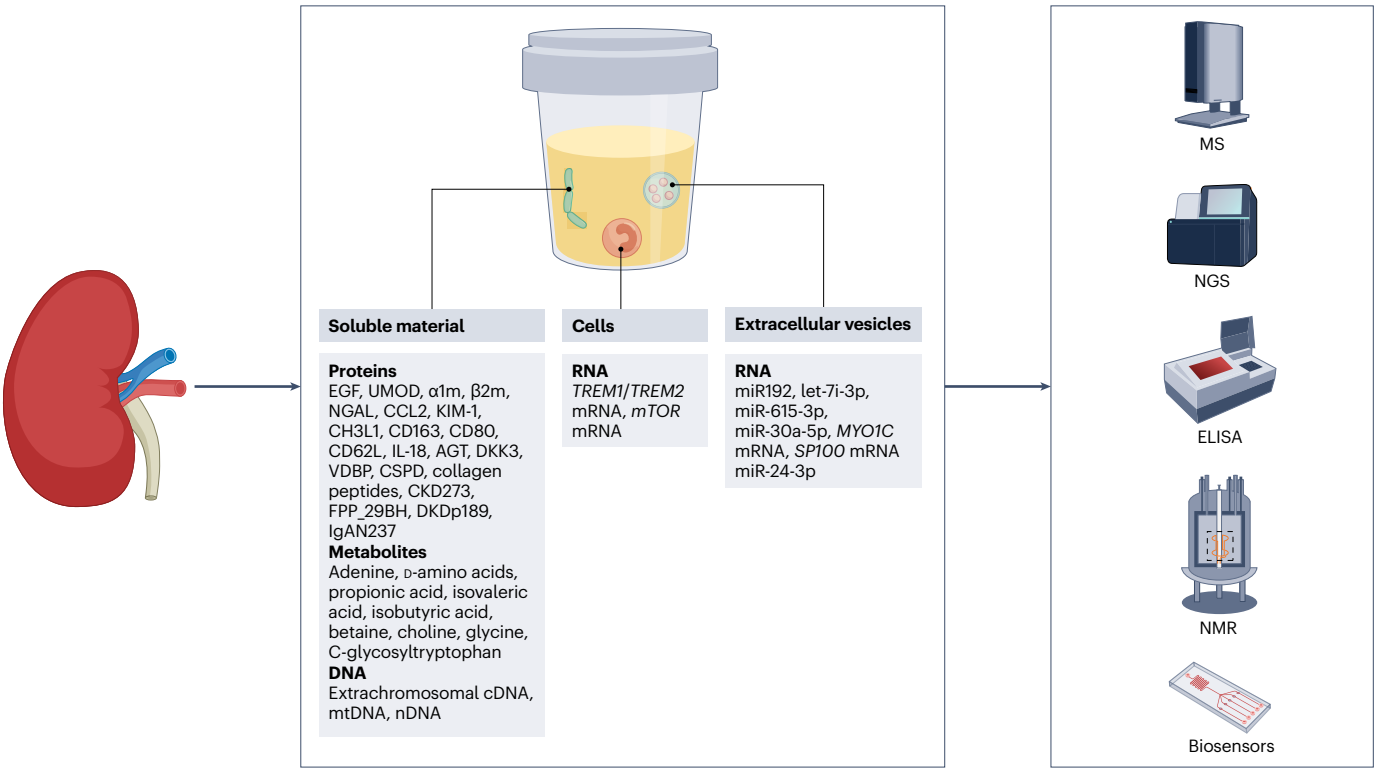

Fig. 1 | Urine components and biomarker analysis. Urine and its components can provide a wealth of information at all molecular levels — proteins, metabolites, nucleic acids — when combined with the use of contemporary analytics tools, including emerging biosensor technologies[140]. Urinary soluble material, extracellular vesicles (exosomes, microvesicles and apoptotic bodies) and cell debris can be released from the nephron (podocytes, tubular cells, glomerular endothelial cells and mesangial cells) and other parts of the urinary tract. As such, urinary components can reflect the filtering, reabsorption and secretion capacity of the kidney, as well as indicate homeostasis, inflammation, fibrosis and damage, while providing mechanistic insights. α1 m, α1-microglobulin; β2 m, β2-microglobulin; AGT, angiotensinogen; CCL2, chemokine (C-C motif) ligand 2 (monocyte chemoattractant protein 1); CD80, cluster of differentiation 80; CD163, urinary soluble cluster of differentiation 163; CD62L, L-selectin; cDNA, circular DNA; CH3L1, chitinase 3-like protein 1; CKD273, 273 peptides, mainly fragments of collagens, uromodulin and pro-inflammatory proteins; CSPD, cathepsin D; DKDp189, 189 peptides, mainly fragments of collagens and A1 anti-trypsin; DKK3, dickkopf 3; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; FPP_29BH, 29 peptides, mainly fragments of collagens; IgAN237, 237 peptides, mainly fragments of collagens, haemoglobin, pro-inflammatory proteins; KIM-1, kidney injury molecule 1; miR-192, let-7i-3p, miR-24-3p, miR-615-3p, miR-30a-5p, types of microRNAs; MS, mass spectrometry; mtDNA, mitochondrial DNA; mTOR, mammalian target of rapamycin; MYO1C, myosin 1C; nDNA, nuclear DNA; NGAL, neutrophil gelatinase-associated lipocalin; NMR, nuclear magnetic resonance; SP100, speckled 100 nuclear antigen; TREM1, triggering receptor expressed on myeloid cells-1; TREM 2, triggering receptor expressed on myeloid cells-2; UMOD, uromodulin; VDBP, vitamin D binding protein.

The pathophysiology of CKD comprises a continuum of accumulated molecular, functional and histological alterations[3,4,9]. Multifactorial cellular injury, particularly of genetic and environmental origin, initiates kidney damage, resulting in oxidative stress, mitochondrial dysfunction, increased intrarenal production of angiotensin II and inflammation, culminating in kidney necrosis and fibrosis.

Hyperglycaemia is one of the best-characterized insults that can promote CKD development[18]. The excessive demand for glucose reabsorption increases the energy needs of proximal tubular cells, resulting in oxygen overconsumption and free-radical production. The pleiotropic consequences of these alterations lead to cumulative irreversible molecular modifications and mitochondrial dysfunction, as well as activation of pro-inflammatory and pro-fibrotic nuclear factor-κB (NF-kB), Janus kinase (JAK)–signal transducer and activator of transcription 3 (STAT3), hypoxia-inducible factor 1α (HIF1α) and AMP-activated protein kinase (AMPK)[9]. Collectively, these alterations fuel anaerobic glycolysis, aberrant cell growth and tissue scarring. Hyperglycaemia also increases the synthesis of angiotensin II and mineralocorticoid receptor (mediator of aldosterone function), which in turn promote fibroblast proliferation and further accelerate kidney fibrosis[9,19].

These molecular alterations in the kidney tissue are reflected in urine and can be mapped in detail with currently available analytical methods (Fig. 1). Below, we review urinary alterations at various molecular levels that occur during CKD development and progression, followed by an overview of representative studies of key biomarker applications (diagnosis, prognosis and monitoring of disease progression and responses to treatment) (Fig. 2).

DNA biomarkers

Nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) biomarkers have been investigated in CKD. The study of mtDNA is of special interest, given the central role of mitochondria in CKD development and progression. Excretion of mtDNA in urine is attributed to kidney, ureter and

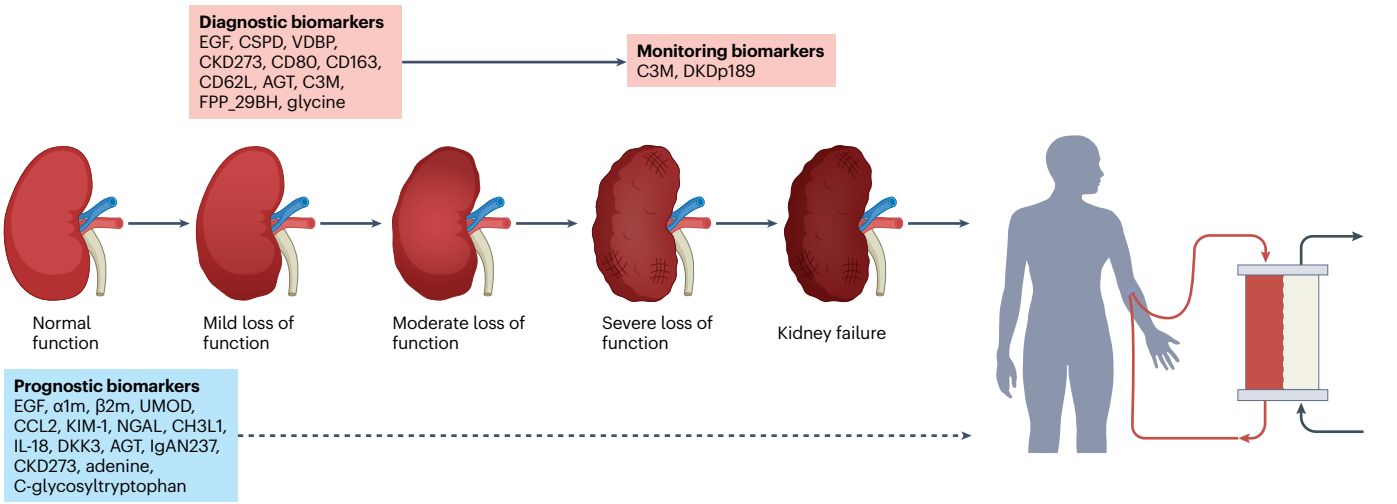

Fig. 2 | Main types of CKD biomarkers. Diagnostic biomarkers can facilitate chronic kidney disease (CKD) detection and its phenotyping, including identification of disease aetiology. Once CKD has been diagnosed, prognostic biomarkers can offer risk estimates for disease progression, whereas monitoring biomarkers reflect disease progression. Both prognostic and monitoring biomarkers can be used to guide patient management and define the optimal treatment course, including the initiation of drug treatment or the implementation of lifestyle modifications that can mitigate the risk of disease progression[141]. Representative markers in soluble urine material for specific areas of application include: incident CKD: EGF, CCL2, α1m, KIM-1, glycine; early diabetic nephropathy: CSPD, VDBP, CKD273; differential diagnosis of aetiologies: CD80, combinations of collagen peptides; active lupus nephritis: CD163, CD62L, EGF; detection of haemodynamic changes, fibrosis: AGT, C3M, FPP_29BH; CKD progression (including diabetic nephropathy): EGF, CCL2, DKK3, AGT, IL-18, UMOD, adenine, C-glycosyltryptophan, IgAN237 (progression of IgA nephropathy), CKD273; progression to CKD from AKI: EGF, CCL2, CH3L1, UMOD. α1 m, α1-microglobulin; β2m, β2-microglobulin; AGT, angiotensinogen; C3M, fragment of collagen III; CCL2, chemokine (C-C motif) ligand 2 (monocyte chemoattractant protein 1); CD163, urinary soluble cluster of differentiation 163; CD62L, L-selectin; CD80, cluster of differentiation 80; CH3L1, chitinase 3-like protein 1; CKD273, panel consisting of 273 peptides, mainly fragments of collagens, uromodulin, and pro-inflammatory proteins; CSPD, cathepsin D; EGF, epidermal growth factor; DKDp189, panel of 189 peptides, mainly fragments of collagens and A1 anti-trypsin; Dkk3, dickkopf 3; FPP_29BH, panel based on 29 peptides, mainly fragments of collagens; IgAN237, panel consisting of 237 peptides, mainly fragments of collagens, haemoglobin, pro-inflammatory proteins; IL-18, interleukin-18; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin; UMOD, uromodulin; VDBP, vitamin D binding protein.

bladder damage. Significant increases in urinary mtDNA copy number have been observed in diabetic kidney disease (DKD), IgA nephropathy (IgAN), antineutrophil cytoplasmic antibody (ANCA)-vasculitis and in kidney-allograft rejection (reviewed in Feng et al.[20]). An analysis of urinary-cell-free mtDNA and nDNA in people with CKD (a substantial fraction of participants had DKD; 11–54% depending on CKD stage) revealed that, over 6 months of follow-up, individuals with stable eGFR ($n = 53$) had lower urinary baseline levels of both DNA types than those who experienced a decrease in eGFR ($n = 70$). The area under the curve (AUC) was 0.685 for mtDNA and 0.730 for nDNA, and both DNA levels correlated positively with UACR[21]. However, in another study limited to people with diabetic nephropathy (DN; $n = 92$), mtDNA in urinary supernatant, urinary sediment or kidney tissue was not significantly associated with fatal events or the need for dialysis within 2 years of follow-up[22]. Nonetheless, urinary supernatant mtDNA correlated positively with interstitial fibrosis (detected based on silver staining; $R = 0.300$) and negatively with intra-renal mtDNA ($R = 0.453$)[22].

In addition to mtDNA and nDNA, urinary extrachromosomal circular DNA (cDNA; defined as circular non-plasmid DNA originating from but not part of chromosomal DNA) was compared in people with advanced CKD ($n = 21$) and healthy individuals ($n = 28$)[23]. Extrachromosomal cDNA was significantly higher in advanced CKD, which was attributed to increased apoptosis and leakage from injured kidney tissue or to glomerular losses due to malfunctioning of the filtration barrier. Overall, analyses of DNA in urine have mostly been performed using relatively small cohorts and despite having biological interest, the biomarker value of these findings remains uncertain.

RNA biomarkers

To increase their specificity, changes in urinary RNA levels frequently rely on omics analyses of urinary extracellular vesicles (EVs), which include exosomes, microvesicles and apoptotic bodies, and are mainly secreted by podocytes, tubular cells, glomerular endothelial cells and mesangial cells[24,25]. Urinary exosomal microRNAs (miRNAs; that is, small non-coding RNAs 18–22 nucleotides long) have been associated with and have early diagnostic value in DKD (reviewed in Wen et al.[26] and Lu et al.[27]). Examples include miR-192, let-7i-3p, miR-24-3p, miR-27b-3p and miR-15b-5p, which have all been associated with early microalbuminuric type 2 diabetes mellitus (T2DM)[28]. Among the targets of these miRNAs are members of the WNT–β-catenin signalling pathway, which is reportedly involved in kidney homeostasis[29,30]. In addition, miR-30a-5p was reported to be associated with macroalbuminuria, presumably reflecting severe kidney damage[28]. Exosomal levels of miRNA-615-3p, which is involved in insulin-like growth factor-2 (IGF-2) regulation, were increased in samples from people with DKD compared with those from people with T2DM without DKD, or healthy individuals. miRNA-615-3p levels also correlated positively with serum levels of cystatin C and transforming growth factor-β1 (TGFβ1), and negatively with eGFR. A higher diagnostic accuracy was obtained when combining urinary exosomal miR-615-3p with UACR, compared

with UACR alone[31]. Nevertheless, the study was insufficiently powered (n = 83) to allow strong conclusions to be drawn about the biomarker value of miR-615-3p. Linked to experimental complexity, follow-up validation studies on exosomal miRNAs are generally scarce, which limits clinical implementation.

The total RNA content of urinary EVs — mRNAs, long non-coding RNAs, circular RNAs and miRNAs — was characterized in 12 individuals with biopsy-proven DN and compared with that of individuals with T2DM without evidence of kidney damage (24-hr urinary albuminuria <30 mg)[32]. Bioinformatics analysis of RNAs differentially expressed in DN indicated their predominant involvement in inflammatory and apoptotic processes. The urinary exosomal myosin 1 C (*MYO1C*) mRNA, which codes for an actin-based podocyte protein that interacts with nephrin and is involved in TGFβ signalling, and the mRNA coding the speckled 100 (SP100) nuclear antigen, which has a role in immune responses, were increased in DN compared with T2DM without DN. Using single-cell and Nephroseq datasets, those findings were validated in kidney tissue when comparing DN versus T2DM without DN, or healthy individuals[32].

In urinary-cell sediments, changes in mRNA levels of triggering receptor expressed on myeloid cells 1 (*TREM1*) and *TREM2*, which are primarily expressed on monocyte-derived cells and are involved in immunomodulation and inflammatory-cell differentiation, were associated with CKD and kidney fibrosis[33]. Urinary *TREM1*-to-*TREM2* mRNA ratio was decreased in people with CKD (n = 77) compared with healthy individuals (n = 15), and correlated positively with eGFR, and negatively with serum creatinine and cystatin C. The decrease was more pronounced in people with moderate-to-severe fibrosis based on Masson trichrome staining of biopsy tissue compared with people with mild or without kidney fibrosis (sensitivity of 86.4%, specificity of 81.8%), and the urinary *TREM1*-to-*TREM2* mRNA ratio correlated negatively with tubulointerstitial fibrosis and glomerular sclerosis scores. Accordingly, the expression of TREM1 was significantly lower than that of TREM2 in kidney tissue with moderate-to-severe fibrosis. Despite this suggested association with fibrosis, large follow-up studies with technical standardization are needed to confirm the biomarker value of these mRNAs.

In IgAN, mammalian target of rapamycin (*mTOR*) mRNA levels in urinary sediment were lower in patients (n = 154) than in healthy individuals (n = 61), correlated negatively with serum creatinine, 24 h proteinuria and cystatin C, and positively with eGFR[34]. A multi-variate logistic regression analysis revealed negative correlations with a tubulointerstitial fibrosis score, as well as differences between cases of severe fibrosis and cases of mild or moderate fibrosis[34]. Overall, many associations between urinary RNAs and CKD have been reported, yet their biomarker value remains to be defined.

Protein biomarkers

Multiple urinary proteins have been studied for their association with CKD phenotypes, prognosis and responses to therapy, including in several large-scale studies for specific contexts of use (Tables 1–3, Supplementary Tables 1–3). In this section, we present findings on protein biomarkers and their associations with kidney-tissue biology (Fig. 3).

Uromodulin (UMOD; also known as Tamm–Horsfall protein) is the most abundant urinary protein and is excreted by epithelial cells of the loop of Henle (~90%) and the distal convoluted tubule (~10%)[35]. UMOD is also released into plasma, where its levels are ~1,000 times lower than those detected in urine (daily kidney excretion range 50–100 mg[36]). Although its exact role remains unclear, UMOD has been associated with regulation of sodium, potassium, calcium and magnesium transporters and channels, regulation of blood pressure, protection against kidney-stone formation and urinary-tract infections, and immunomodulatory and anti-inflammatory effects (reviewed in Thielemans et al.[35] and Nanamatsu et al.[37]). These pleiotropic functions might result from the presence of polymeric and non-polymeric forms of UMOD[37]. *UMOD* variants have also been linked to CKD risk in genome-wide association studies. Urinary UMOD is thought to reflect tubular secretion capacity, nephron mass and kidney function[38]. Data from the Swiss Kidney Project on Genes in Hypertension (SKIPOGH) and Cohorte Lausannoise (CoLaus) cohorts[39], and the CARTaGENE cohort from Canada[40] suggested positive associations of urinary UMOD (24 h secretion and/or spot urine levels) with urinary sodium, as well as the eGFR. In addition, when comparing groups of people with increasing duration of diabetes (duration of <1 year; 1–4 years; 5–9 years; 10–14 years), UMOD was increasingly excreted, although this excretion declined and correlated negatively with albuminuria if diabetes duration exceeded 15 years[35,41,42]. Given its role in tissue regeneration, the diabetes-associated rise in UMOD levels might reflect an upregulation in response to persistent injury driven by hyperglycaemia. However, such an upregulation might be impossible to maintain for longer periods, leading to the reduction observed in persistent diabetes[35]. Despite extensive studies, the value of UMOD as a biomarker is still not defined and is complicated by the presence of different structural isoforms and the limited understanding of their respective functions[37].

Several other markers also reflect kidney dysfunction, tubular injury, inflammation or fibrosis. Urinary α1-microglobulin (α1m) and β2m are indicators of tubular dysfunction as they are normally filtered by the glomeruli and subsequently reabsorbed by the proximal tubules. Consequently, increased excretion of these proteins indicates tubular injury and defects in reabsorptive capacity, and has been associated with CKD progression and mortality[43]. Kidney injury molecule 1 (KIM-1) is a membrane protein acutely (over)expressed in the proximal tubule following kidney injury (reviewed in Song et al.[44]); this protein is characterized by an extracellular segment carrying a variable immunoglobulin domain and a mucin region, and an intracellular tail with a Tyr phosphorylation domain. KIM-1 acts as a receptor for viruses, facilitates the engulfment of apoptotic cells, mediates the release of intracellular calcium and activates various intracellular signalling pathways. Associations of KIM-1 with tubular cell injury have also been suggested based on its role as a receptor for albumin-bound fatty acids in proximal tubular cells; KIM-1 binding triggers pro-inflammatory and pro-fibrotic pathways[45]. Accordingly, increased urinary KIM-1 levels have been linked to inflammation and fibrosis, and result from shedding of the protein extracellular domain, following activation of type I and III membrane matrix metalloproteinases and A disintegrin and metalloproteinases[46].

Urinary angiotensinogen (AGT) is the substrate of renin and a marker of intrarenal renin–angiotensin–aldosterone system activity, which regulates fluid balance and blood pressure[47]. Glomerular injury has been associated with urinary excretion of AGT, which, given the involvement of AGT in blood-pressure regulation, is thought to affect kidney haemodynamics[48] and to have diagnostic and prognostic potential, particularly in cardiorenal syndrome[49].

Multiple immune-system-related proteins have also been explored for their diagnostic and prognostic potential. These include urinary soluble CD163, a transmembrane glycosylated protein excreted by activated M2 macrophages in response to increased inflammation, as well as CD80, which is typically expressed on antigen-presenting cells

Table 1 | Representative studies of urinary markers with potential diagnostic value

Molecule (trend in disease)	Diagnostic application	Cohort	Metrics of performance	Ref.
EGF (decrease)	Incident CKD	PREVEND *n*=4,534; RENIS *n*=1,249	OR from 1.27 (0.99 to 1.62) to 1.72 (1.01 to 2.95) depending on the definition of incident CKD	80
EGF (decrease), CCL2 (increase), α1m (increase), KIM-1 (increase), CH3L1 (increase)	Incident CKD	ARIC *n*=872; MESA *n*=495; REGARDS *n*=493	ARIC: CCL2 HR 1.26 (1.14, 1.39); α1m HR 1.20 (1.07, 1.34), KIM-1 HR 1.16 (1.06, 1.27) MESA: CCL2 HR 1.38 (1.02, 1.86); EGF HR 0.57 (0.35, 0.95) All cohorts combined: α1m HR 1.19 (1.08, 1.31)	81
Glycine (decrease), histidine (decrease)	Incident CKD	Framingham Offspring cohort *n*=193 CKD; ARIC *n*=998, including *n*=149 CKD	Framingham: glycine OR 0.59 (0.43–0.80) Histidine OR 0.65 (0.50–0.85) ARIC: glycine HR 0.82 (0.69–0.99)	82
Cathepsin D (increase)	Early DN in T1DM	JDRF *n*=1,270	OR 1.29 (1.07 to 1.55) of eGFR decline	62
CKD273 (increase)	Early DN in T2DM	PRIORITY *n*=1,775	HR 2.48 (1.80 to 3.42) of microalbuminuria	83
Gd-IgA1 (increase)	Detection of IgAN	Japanese cohort *n*=458; other Asian cohort *n*=143	Correlations with IgAN histological grade (R=0.4108)	87
CD80 (increase)	Detection of MCD	NEPTUNE *n*=104; Mayo Clinic cohort *n*=307	AUC=0.74 (MCD versus FSGS, DN, and IgAN); AUC=0.69 (MCD versus FSGS)	52
Multi-peptide panel	Detection of CKD aetiology	Multiple cohorts *n*=1,850 separated into training (*n*=1,388) and test (*n*=462) sets	86.47%, 82.61%, 63.16% and 90.48% accuracies for differential detection of DN, IgAN, vasculitis and healthy individuals, respectively	89
EGF (decrease)	Active LN	Ohio and Mexican cohorts *n*=224	Correlation with: chronicity index of histological features (*R*=−0.67), glomerular sclerosis (*R*=−0.539), interstitial fibrosis (*R*=−0.654), tubular atrophy (*R*=−0.665)	91
CD163 (increase)	Active LN	Mexican and Ohio State University cohorts *n*=425	AUC=0.998 or 0.980 for active versus inactive LN, depending on the cohort	93
CD163 (increase)	Active kidney vasculitis	Two-centre database *n*=138 ANCA-associated glomerulonephritis separated into inception *n*=101 and validation *n*=37 sets	Combined to serum calprotectin and haematuria sensitivity 78%, specificity 94%, likelihood ratio 13 in discerning activity	94
Selectin (CD62L) (increase)	Active LN	Chinese cohort *n*=255 and US cohort *n*=219	Association with histological activity index, *R*=0.34 or *R*=0.47 depending on the cohort	59
C3M (decrease)	Fibrosis	Single centre, *n*=133 IgAN	AUC=0.81 for advanced versus low to moderate fibrosis, association with fibrosis as a continuous variable (*R*=−0.24)	64
FPP_29BH (increase)	Fibrosis	Single centre *n*=435 CKD separated into discovery *n*=200 and test *n*=235 sets	Correlation with interstitial fibrosis and tubular atrophy (*R*=0.5)	63
AGT (increase)	Cardiorenal syndrome	Japanese community-based cohort *n*=282	Associations with multiple aortic and kidney haemodynamic parameters	48

α1m, α1 microglobulin; AGT, angiotensinogen; ARIC, Atherosclerosis Risk in Communities; AUC, area under the curve; CCL2, CC-chemokine ligand 2; CD80, cluster of differentiation 80; CD163, cluster of differentiation 163; CD62L, selectin; C3M, collagen III fragment marking degradation; CKD, chronic kidney disease; DN, diabetic nephropathy; FPP_29BH, panel of 229 peptides (mainly collagen fragments); CH3L1, chitinase 3-like 1; CKD273, panel of 273 peptides (mainly fragments of collagens, uromodulin and pro-inflammatory proteins); EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; Gd-IgA1, galactose-deficient IgA1; HR, hazard ratio; IgAN, IgA nephropathy; JDRF, Juvenile Diabetes Research Foundation Cohort; KIM-1, kidney injury molecule-1; LN, lupus nephritis; MESA, Multi-Ethnic Study of Atherosclerosis; NEPTUNE, Nephrotic Syndrome Study Network; OR, odds ratio; PREVEND, Prevention of Renal and Vascular End-stage Disease; PRIORITY, Proteomic Prediction and Renin–Angiotensin–Aldosterone System Inhibition Prevention of Early Diabetic Nephropathy in Type 2 Diabetic Patients with Normoalbuminuria; *R*, Spearman or Pearson correlation factor; REGARDS, Reasons for Geographic and Racial Differences in Stroke; RENIS, Renal Iohexol Clearance Group. Reported models and performance are from the independent validation (test) sets and correspond to adjusted (including for eGFR and albuminuria) models, as applicable. Where multiple markers were investigated, only the associations that reached significance are reported.

but in people with minimal change disease is also frequently detected on glomerular epithelial cells and podocytes[50], with attributed effects in the activation of innate and adaptive immunity[51,52], as well as cytoskeletal reorganization and podocyte migration[50]. Another relevant marker is neutrophil gelatinase-associated lipocalin (NGAL), which is secreted by activated neutrophils and also epithelial tubular cells of the distal convoluted tubules. NGAL is involved in inflammatory responses, cell differentiation, proliferation, apoptosis, iron homeostasis and fibrosis[53]; following kidney injury, NGAL shedding increases. The diagnostic and prognostic value of serum and urinary NGAL have been tested extensively, mainly in AKI but also in IgAN and nephrotic syndrome (reviewed in He et al.[54]). Other markers include urinary osteopontin (OPN), a small integrin-binding ligand *N*-linked glycoprotein expressed in tubular cells that, in addition to its role in bone formation, has been associated with kidney inflammation, apoptosis and damage[55,56]. IL-18 is also released into urine by immune cells via the proximal tubule following tubular injury[57]. Similarly, release of chitinase 3-like 1 (CH3L1, also known as YKL-40) expressed in kidney

Table 2 | Representative studies of urinary markers with prognostic potential

Molecular change associated with increased risk	Application	Cohort	Metrics of performance	Ref.
EGF-to-CCL2 ratio (decrease)	DN progression	Joslin Kidney Study *n*=1,032 T2DM with normal eGFR and normo- or albuminuria at baseline	Per 1 quartile increase EGF/CCL2 ratio OR 0.58 (0.46 to 0.74) for eGFR decline	101
EGF (decrease), UMOD (decrease)	DKD progression	VA NEPHRON-D *n*=1,116 with mean eGFR of 56 (s.d. 19) ml/min/1.73 m^2, and median UACR of 840 (IQR 424–1780) mg/g creatinine at baseline	Per two-fold increase (continuous model) from baseline: EGF/creatinine HR 0.68 (0.47, 0.99) UMOD/creatinine 0.85 (0.75, 0.98) for kidney function decline or kidney failure	103
EGF (decrease)	Progression to CKD in individuals with AKI	ASSESS AKI *n*=1,509	Two-fold increase in urinary EGF-to-creatinine ratio at 3 months after hospitalization HR 0.46 (0.39 to 0.55) for development of CKD	104
AGT (increase)	CKD Progression	KNOW-CKD *n*=1,688	Urinary AGT-to-creatinine ratio highest versus lowest quintile: HR 1.53 (1.16 to 2.02) for composite outcome	105
DKK3 (increase)	CKD progression	CARE FOR HOMe CKD *n*=575 STOP-IgAN cohort *n*=153	Association of urinary DKK3-to-creatinine ratio >4,000 pg/mg with mean eGFR decline of 7.6% (−10.8 to −4.3%) over 12 months	106
UMOD (decrease)	CKD progression	Cardiovascular Health Study (community dwelling adults *n*=3,313); subcohorts: CKD progressors and non-progressors *n*=423, random subcohort *n*=958	Increase in UMOD (by 19.7 µg/ml) OR 0.77 (0.62 to 0.96) for eGFR decline; HR 0.90 (0.83 to 0.98) for mortality	107
NGAL (increase), KIM-1 (increase), IL-18 (increase), CCL2 (increase), CH3L1 (increase)	CKD progression	VA NEPHRON-D *n*=1,135	Two-fold increase in CCL2: HR 1.37 (1.15, 1.62); CH3L1: HR 1.07 (1.00, 1.13) for kidney function decline	108
CCL2 (increase), CH3L1 (increase), UMOD (decrease)	Progression to CKD post-AKI	ASSESS-AKI cohort *n*=1,538, marker measurement at 3 months post-discharge	Lowest versus highest quartiles: CCL2: eGFR decline by 8.0% (7.1 to 9.0) versus 17.8% (16.7 to 18.8); CH3L1: eGFR decline by 7.9% (7.0 to 8.9) versus 21.7% (20.6 to 22.7); lowest versus highest quartile: UMOD: eGFR decline by 19.8% (18.8 to 20.8) versus 9.9% (9.0 to 10.9)	110
KIM-1 (increase), CCL2 (increase), CH3L1 (increase), IL-18 (increase)	CKD progression	SPRINT *n*=2,428 patients with CKD	Highest versus lowest quartiles: KIM-1 HR 2.84 (1.31 to 6.17), CCL2 HR 2.43 (1.13 to 5.23), CH3L1 HR 1.95 (1.08 to 3.51) for composite outcome; IL-18 β −0.22 (−0.36 to −0.08) linear association with eGFR decline	57
EGF (decrease)	LN progression	Mexican and Ohio cohorts *n*=120 active LN EGF measurement at the time of LN flare	Urinary EGF-to-creatinine ratio association with time to DSCr: HR 0.88 (0.77–0.99); urinary EGF-to-creatinine ratio <5.3 ng/mg 81% sensitivity 77% specificity for DSCr within 2 years	91
IgAN237 (increase)	IgAN progression	PersTIgAN *n*=209 patients with IgAN separated into training set *n*=94 and test set *n*=46	AUC=0.72 for IgAN progressors versus non-progressors; compared with the use of clinical parameters alone, AUC increased from 0.72 to 0.89 with addition of IgAN237	112
		Multi-centric validation cohort *n*=103 IgAN	IgAN237 negatively associated with ±180 days eGFR slopes (R=−0.310)	113
Factor analysis tubular function (EGF, ADMA, SDMA; decrease), tubular damage (α1m, KIM1, CCL2; increase)	CKD progression	CRIC *n*=701; REGARDS *n*=555	Per one-standard deviation, CRIC: tubular function HR 0.36 (0.25–0.52); tubular damage HR 1.45 (1.18–1.78); REGARDS: tubular function HR 0.81 (0.47–1.39]; tubular damage HR 1.12 (0.73–1.72)	114
C-glycosyltryptophan (increase)	CKD progression	German Chronic Kidney disease Study *n*=5,087	HR 1.43 (1.27–1.61) for kidney failure; HR 1.40 (1.27–1.55) for kidney failure and AKI; 1.47 (1.33–1.63) for mortality	115
Adenine (increase)	Progression to kidney failure	CRIC *n*=904; SMART2D *n*=309; American Indian Study *n*=54	Highest tertile of adenine to creatinine HR 1.57 (1.18 to 2.10) for kidney failure (CRIC); HR 2.39 (1.08 to 5.29) for non-macroalbuminuric subgroup (SMART2D); HR 4.47 (1.53 to 13.06) non-macroalbuminuric subgroup (American Indian)	117

α1m, α1 microglobulin; β, slope in linear regression; β2m, β2-microglobulin; ADMA, urine asymmetric dimethylarginine; AGT, angiotensinogen; AKI, acute kidney injury; ASSESS AKI, Assessment, Serial Evaluation, and Subsequent Sequelae of Acute Kidney Injury; AUC, area under the curve; CCL2, CC-chemokine ligand 2; CH3L1, chitinase 3-like 1; CKD, chronic kidney disease; CRIC, Chronic Renal Insufficiency Cohort; CV, cardiovascular; DKK3, dickkopf 3; DSCr, doubling of serum creatinine; DN, diabetic nephropathy; EGF, epidermal growth factor; HR, hazard ratio; IgAN, IgA nephropathy; IgAN 237, panel of 237 peptides (mainly fragments of collagen, haemoglobin, pro-inflammatory proteins); IL-18, interleukin 18; KIM-1, kidney injury molecule-1; KNOW-CKD, Korean Cohort Study for Outcomes in Patients With Chronic Kidney Disease; NGAL, neutrophil gelatinase-associated lipocalin; OR, odds ratio; PersTIgAN, Personalized Treatment in IgA Nephropathy; REGARDS, Reasons for Geographic and Racial Differences in Stroke; SDMA, urine symmetric dimethylarginine; SMART2D, Singapore Study of Macro-angiopathy and Micro-vascular Reactivity in Type 2 Diabetes; SPRINT, Systolic Blood Pressure Intervention Trial; T2DM, T2 diabetes mellitus; UMOD, uromodulin; VA Nephron-D, Veterans Affairs Diabetes in Nephropathy. Composite outcome: >50% decline in eGFR, doubling of serum creatinine or kidney failure (AGT); 50% eGFR decline or kidney failure (CCL2, KIM-1, CH3L1). Reported models and performance are from the independent validation (test) sets and correspond to adjusted (including for eGFR and albuminuria) models, as applicable. Where multiple markers were investigated, only the associations that reached significance are reported.

Table 3 | Representative studies of urinary markers with therapeutic response monitoring potential

Molecular change associated with positive response to treatment	Application	Sample size	Metrics of performance	Ref.
KIM-1 (decrease), CCL2 (decrease)	Effect of canagliflozin	CANVAS *n*=763	At year 1 canagliflozin versus placebo: CCL2 decrease by 18.1% (8.9, 26.4) KIM-1 decrease by 30.9% (23.0, 38.0)	118
KIM-1 (decrease), IL-1β (decrease), mtND1 copy number (decrease)	Effect of dapagliflozin	Single centre *n*=54 CKD; *n*=20 healthy	At month 6, reduction of all markers; correlations with eGFR KIM-1 *R* −0.358, IL-1β *R* −0.371	119
DKDp189 (decrease)	Response to ARB in diabetes	DC-REN *n*=199, discovery set; PRIORITY *n*=468; DIRECT-Protect 2 *n*=194, validation sets	AUC=0.6 (PRIORITY), AUC=0.63 (DIRECT-Protect 2) for progressors (uncontrolled) versus non-progressors (controlled) based on multiple eGFR equations	120
C3M (increase)	Nephroprotective impact of GLP-1 R agonists	AWARD-7 trial *n* = 330	C3M change from baseline to week 52 higher in the dulaglutide versus insulin glargine group; positive correlations with eGFR in both groups	121

ARB, angiotensin II receptor blocker; AUC, area under the curve; AWARD-7, Assessment of Weekly Administration of LY2189265 (dulaglutide) in Diabetes-7; CANVAS, Canagliflozin Cardiovascular Assessment Study; CCL2, CC-chemokine ligand 2; C3M, collagen III fragment marking degradation; DC-REN, Drug Combinations for Rewriting Trajectories of Renal Pathologies in Type II Diabetes; DIRECT-Protect 2, Diabetic Retinopathy Candesartan Trial; DKDp189, panel of 189 peptides (mainly fragments of collagens, A1-anti-trypsin); eGFR, estimated glomerular filtration rate; GLP-1R, glucagon-like peptide 1 receptor; KIM-1, kidney injury molecule-1; mtND1, mitochondrial DNA nicotinamide adenine dinucleotide dehydrogenase subunit- 1; PRIORITY, Proteomic Prediction and Renin Angiotensin Aldosterone System Inhibition Prevention of Early Diabetic Nephropathy in Type 2 Diabetic Patients with Normoalbuminuria.

immune cells, and CC-chemokine ligand 2 (CCL2, also known as MCP-1), which is a key mediator of kidney inflammation owing to its expression in the endothelial glycocalyx and its monocyte chemoattractant effect[3], are thought to reflect tubular injury. In addition, CD62L (also known as L-selectin) — expressed on circulating leukocytes — mediates cell adhesion and facilitates inflammatory-cell infiltration. Single-cell RNA-sequencing data from kidney cells and immune cells infiltrating the kidneys from lupus nephritis (LN) biopsy tissue indicate that CD26L is primarily detected in infiltrating B cells[58]. Accordingly, CD62L urinary levels increase in autoimmune diseases, particularly in active LN compared with systemic lupus erythematosus (SLE) without kidney invlolvement[59].

Urinary lysosomal proteins have also been associated with CKD. *N*-acetyl beta-D-glucosaminidase (NAG) is a lysosomal glycosidase commonly expressed in proximal tubule epithelial cells. Given that NAG cannot pass through the glomerular filtration barrier, urinary NAG is an explicit marker of tubular injury[60]. Cytosolic cathepsin D levels can rise in the context of lysosomal stress and, in this way, contribute to degradation of extracellular matrix components, which, in turn, can affect glomerular permeability[61]. Tubular levels of cathepsin D are high in DKD, possibly to meet the increased demand for albumin degradation in urine[61,62]. Increased urinary cathepsin D excretion has been linked to tubulointerstitial damage and eGFR decline[62].

Collagens have been studied for their biomarker potential in kidney fibrosis, either individually or as components of multi-parametric classifiers. Turnover fragments of collagen III (collagen 3 M, collagen 3 C and pro-collagen 3), and fragments of collagen I have been investigated as markers of kidney fibrosis and early CKD, as well as predictive markers of CKD prognosis and treatment outcomes[63,64].

The presence of various additional signalling proteins in urine might have potential biomarker value. Dickkopf 3 (DKK3) is a glycoprotein that regulates the WNT–β-catenin signalling pathway; it is expressed and secreted by tubular cells in response to stress, and considered to be an indicator of tubular injury[65]. Epidermal growth factor (EGF) is produced mainly in the loop of Henle and the distal convoluted tubule. EGF has been attributed roles in haemodynamics through activation of ion channels (such as calcium or magnesium channels) downstream of its receptor, as well as contributing to cell growth and tissue repair via activation of diverse signalling pathways (including the PI3K–Akt–PTEN–mTOR and JAK–STAT pathways). Although EGF is hardly detectable in plasma, it can be detected in the urine of healthy individuals, with urinary EGF levels decreasing in kidney disease. EGF expression in the kidney and its subsequent excretion in urine have prompted multiple studies highlighting the diagnostic and/or prognostic value of urinary EGF (reviewed in Cortvrindt et al.[66]).

Urinary vitamin D binding protein (VDBP) is part of the albumin family and is involved not only in the transport of vitamin D and its metabolites, but also in macrophage activation. Several reports suggest that increased urinary VDBP levels are an early marker of DKD[67].

Metabolite biomarkers

Multiple urinary metabolites, derived from nutrient uptake and/or cell or microbial metabolism, are altered in CKD. Metabolomic strategies have highlighted the pathophysiological role of uraemic toxins (reviewed in Glorieux et al.[68] and Rosner et al.[69]) and their potential biomarker value (for example, for oxalate[70]). A systematic review[71] found that the ten most frequently reported metabolites in urine include (in addition to creatinine): trimethylamine *N*-oxide (TMAO), citric acid, hippuric acid, phenylalanine, asymmetric dimethylarginine (ADMA), glutamine, taurine, 3-hydroxylsovaleric acid and *p*-hydroxyphenylacetic acid. These molecules are involved in multiple pathways and biological processes, including glutamine, glutamate, glyoxylate and homocysteine metabolism, the tricarboxylic acid (TCA) cycle, protein methylation, mitochondrial homeostasis and osmolality preservation via the arginine vasopressin–cyclic adenosine monophosphate–aquaporin 2 pathway[72]. Among amino-acid metabolites, the rare D-enantiomers, including D-serine, D-alanine, D-proline and D-asparagine, have attracted special attention as they are strictly regulated by the kidney, providing an alternative to GFR estimates[73]. In that context, the simultaneous assessment of blood and urinary levels of D-amino acids might provide a more accurate indication of kidney function than conventional creatinine-based GFR estimates[73]; nevertheless, lack of standardization of the

quantification strategies and cost determination remain barriers to clinical implementation.

Changes in lipid metabolism of relevance to CKD are also reflected in urine. Direct metabolomic analysis of urine samples from people with early autosomal-dominant polycystic kidney disease compared with those of healthy individuals revealed increases in many lipidic compounds including eicosapentaenoic, linoleic and stearolic acids, ethanolamine, C20:4 anandamide phosphate, and various androgens[74]. Moreover, urinary lipids (mainly 1-palmitoyl-2-arachido noyl-glycerophosphatidylcholine (GPC) and various derivatives) were associated with UACR in a general population cohort, perhaps owing to conjugation to urine albumin and/or excretion via damaged cell membranes[75]. Similarly, the short-chain fatty acids propionic, isovaleric and isobutyric acids were reported at increased levels in urine from people with DKD compared with those with T2DM without kidney disease[76], whereas urinary betaine and choline have been positively associated with CKD progression[76,77].

Collectively, several targeted and untargeted studies have investigated the metabolome of CKD. In some cases, parallel analysis of urine and blood metabolites was conducted[75,78] and provided insights into the effect of the disease on filtration and/or tubular secretion. For metabolites detected in both blood and urine, abundance correlations ranging from −0.23 to 0.94 were reported[75].

Although valuable functional information has been collected, providing insights into pathophysiology and allowing characterization of some metabolites as uraemic toxins[69], the biomarker value of these findings remains insufficiently defined. This uncertainty is related to the frequent use of small case–control studies, lack of validation, the vast complexity of the metabolome, as well as the influence of a large array of confounding factors, ranging from sample collection, and processing and storage strategies, to the specific features of each cohort, for example, general population versus disease-specific groups, and the age, comorbidities, microbiome and lifestyle of each group.

Application value of biomarkers

Here we summarize representative large-scale evidence on the potential applications of urinary biomarkers in CKD, grouped according to diagnostic, prognostic (focusing on CKD progression, defined as kidney failure, mortality, and/or composite endpoints associated with eGFR changes) or therapeutic monitoring[79] validity. We acknowledge that multiple additional biomarkers and studies other than those discussed here have been reported. The studies included here were selected based on their large or multi-cohort and/or prospective study design, and/or their relevance to the characterization of the disease pathophysiology with impact on disease progression and management.

Diagnostic markers

Multiple urinary markers have been attributed diagnostic potential in CKD (Table 1, detailed in Supplementary Table 1). Below, we focus on findings according to the main clinical application of the marker, for example, disease detection in the general population and early diagnosis (such markers might also have prognostic value), differential diagnosis of CKD aetiologies and evaluation of disease severity.

The biomarker value of urinary EGF has been addressed in multiple studies. Urinary EGF correlates with tissue *EGF* mRNA and decreased levels are considered indicative of tubular atrophy and interstitial kidney fibrosis[66]. Based on data from the Renal Iohexol Clearance Survey (RENIS) and the Prevention of Renal and Vascular End-stage Disease (PREVEND) cohorts, the combination of urinary EGF, eGFR and UACR was proposed to detect incident CKD in the general population[80]. Similar associations of decreased urinary EGF with incident CKD have also been demonstrated in the Multi-Ethnic Study of Atherosclerosis (MESA) cohort[81] involving individuals at a low risk of CKD (specifically, participants without diabetes and an eGFR ≥60 ml/min/1.73 m^2). However, this association could not be replicated in two additional similarly low-risk cohorts (Atherosclerosis Risk in Communities (ARIC), and Geographic and Racial Differences

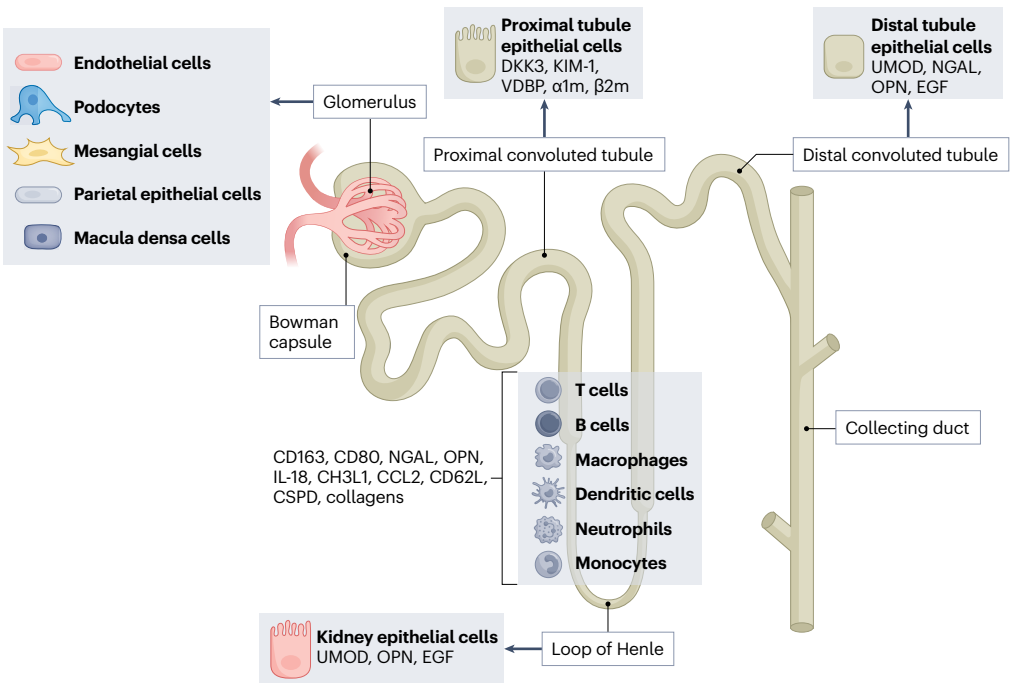

Fig. 3 | Main cells and tissue compartment origin of frequently reported urinary protein biomarkers. For most depicted markers, chronic kidney disease (CKD) and its comorbidities are associated with an increase in urinary levels of these proteins. Uromodulin (UMOD), epidermal growth factor (EGF) and many reported collagen fragments included in peptide panels are an exception, with observed decreases in disease. Identification of the biomarker origin can be indicative of the main site of injury and dysfunction and have therapeutic implications. a1m, α1-microglobulin; β2m, β2-microglobulin; CCL2, CC-chemokine ligand 2; CD163, urinary soluble cluster of differentiation 163; CD62L, L-selectin; CH3L1, chitinase 3-like 1; CSPD, cathepsin D; DKK3, dickkopf 3; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin; OPN, osteopontin; VDBP, vitamin D binding protein.

in Stroke (REGARDS))[81]. Among the other markers tested in the same study[81] (CCL2, KIM-1, CH3L1 and α1m), increased levels of CCL2 also associated with an increased risk of incident CKD in both the MESA and ARIC cohorts, whereas a meta-analysis of all three cohorts reported a statistically significant association of α1m[81]. Although none of the tested markers performed consistently throughout all cohorts, to some extent owing to cohort differences in clinical and pathological backgrounds, these studies indicate that early alterations in kidney tissue (tubular cell damage and repair (reflected by KIM-1 and EGF); immune-cell infiltration (CCL2) and impaired reabsorptive capacity (α1m) are reflected in early urinary changes.

Associations with incident CKD have been investigated in metabolomic studies. Urine analysis from individuals who developed CKD (eGFR <60 ml/min/1.73 m^2) after a mean follow-up of 9.7 years versus a matched group of individuals without CKD from the Framingham Offspring cohort showed decreased levels of urinary glycine and histidine in the group with CKD, with negative associations with incident CKD in multivariate analysis. The association of glycine was further confirmed in the ARIC cohort[82].

In DKD, markers with potential application in early detection include VDBP, cathepsin D and the CKD273 classifier. In a meta-analysis, urinary VDBP was increased in people with normoalbuminuria and diabetes compared with healthy individuals, with further increases in those with microalbuminuria, and still higher values in those with macroalbuminuria[67]. VDBP also correlated positively with UACR, serum creatinine, blood urea nitrogen (BUN) and haemoglobin A1C (HbA1C). However, the heterogeneity among analysed studies and the frequent lack of adjustment for confounders underscore the need for additional studies. In a multi-institution case–control study involving four cohorts of people with type 1 diabetes, urinary cathepsin D was increased in cases of rapid eGFR decline[62]. The study involved large discovery and validation cohorts (Table 1, Supplementary Table 1) with analyses performed in both kidney tissue and urine; the data suggested an increase of cathepsin D levels in proximal tubules in DN, and urinary and tissue cathepsin D were associated with tubulointerstitial morphological changes[62]. In the prospective PRIORITY trial, the CKD273 classifier — a 273 peptide panel detected by capillary electrophoresis mass spectrometry — could distinguish, among people with diabetes and normoalbuminuria, those at a high or low risk of DKD development. Within the classifier high-risk group, 28% of participants developed microalbuminuria compared with 9% in the low-risk group over a median follow-up time of 2.51 years[83]. The prognostic biomarker value of the same panel was also demonstrated for rapidly progressing CKD[84]. Of note, the classifier abundance score in people with diabetes and microalbuminuria decreases following treatment with the angiotensin II receptor blocker irbesartan[85].

In the context of the differential diagnosis of CKD, the discriminatory value of galactose-deficient IgA1 (Gd-IgA1), formed during the early phase of IgAN development[86], represents a potential specific marker of the disease. Gd-IgA1 was assessed in three Asian cohorts for its ability to differentiate IgAN from other kidney diseases; urinary Gd-IgA1 was increased in IgAN, although significant overlaps with other diseases were found, compromising its specificity[87]. In addition, urinary Gd-IgA1 correlated positively with histopathological disease severity. Similar results were reported in a 2023 meta-analysis, although specific cut-off values were not presented, presumably owing, at least in part, to high heterogeneity[88]. Lack of a defined optimal Gd-IgA1 quantification method remains a key implementation challenge.

The biomarker potential of urinary CD80 in the differential diagnosis of minimal-change disease (MCD) was suggested in a study involving people with various nephrotic diseases and healthy individuals[52]. Urinary CD80-to-creatinine ratios were higher in people with MCD than in healthy individuals or those with several other diseases, including focal segmental glomerulosclerosis (of note, early focal segmental glomerulosclerosis is often confused with MCD). Urinary levels of CD80 correlated positively with proteinuria and were also higher during relapses than in periods of remission. This discriminatory potential of urinary CD80, along with its association with specific immunological phenotypes of MCD and response to immunosuppressants, needs further clinical investigation.

Multiple peptide classifiers have been assessed in the differential diagnosis of CKD aetiologies[89,90]. Applying support vector machines (machine-learning model) and the statistical pipeline uniform manifold approximation and projection to reduce peptide data dimensionality enabled the positioning of each CKD aetiology (IgAN, DN and vasculitis) and controls in a 3D space as distinct clusters[89]. Further assessment of the differential diagnostic potential of this approach in clinical studies might reveal added value in enabling early diagnosis and timely initiation of optimal treatment.

Discrimination of active disease is another biomarker target. EGF-to-creatinine ratio was lower in people with active LN than in those with active non-renal SLE, or inactive or mildly active SLE, correlating positively with eGFR at the time of flare and negatively with the kidney biopsy histological chronicity index[91]. Similarly, urinary soluble CD163 was significantly higher in people with active LN than in those with extrarenal or inactive SLE; in addition, CD163 levels correlated positively with disease severity (defined by high disease activity scores, urine protein-to-creatinine ratio and levels of anti-double-stranded DNA antibodies)[92,93]. The same marker (CD163), in combination with serum calprotectin and haematuria, had sensitivity, specificity and likelihood ratios of 78%, 94% and 13, respectively, when used to discriminate active and remission phases in ANCA vasculitis[94].

Similarly, in a cross-sectional study of two independent cohorts from China and the USA, levels of urinary CD62L were higher in people with active LN versus people with inactive LN, active or inactive non-renal SLE, CKD of causes other than SLE, or healthy individuals. Urinary CD62L levels were also associated with histopathological LN indices describing semi-quantitatively active and chronic lesions[95]; positive associations with histological activity index were particularly pronounced[59]. Furthermore, in a small subset (n = 20) of participants with at least 6 months of follow-up, the addition of urinary CD62L levels improved multivariate models predicting a high chronicity index, and a decrease in CD62L was observed in people with complete remission of LN, suggesting predictive and/or monitoring potential[59].

Urinary collagen fragments and classifiers have been used to estimate the degree of fibrosis in various kidney diseases. A urinary classifier consisting of 29 peptides, mainly of collagen origin, correlated with interstitial fibrosis and tubular atrophy, and differentiated individuals with fibrosis from those without fibrosis both in the discovery and in the validation set[63]. In another study[64], fragments of collagen III (C3M and C3C marking degradation, and PRO-C3 reflecting synthesis) were measured in both urine and serum of people with IgAN the day before a kidney biopsy. All quantified markers (direct measurements or amounts normalized for urinary creatinine) were associated with serum creatinine and eGFR. Urinary C3M-to-creatinine ratio was negatively associated with advanced fibrosis, independently

of the other fragments, proteinuria and serum creatinine, and provided added value to serum creatinine when modelled to indicate fibrosis burden[64]. These observed associations of fibrotic markers merit further clinical validation, as they could facilitate early identification of fibrosis, support preventive measures and guide disease management.

In adults >50 years old, including people with CKD, urinary AGT correlated positively with pulsatile measures of kidney blood flow as well as aortic blood pressure and pulse-wave velocity, plasma N-terminal pro brain natriuretic peptide (NT-proBNP) and serum fibroblast growth factor 23 (FGF23)[48]. Age, sex, BMI, eGFR and medication did not affect these associations, suggesting that urinary AGT reflects kidney and aortic haemodynamics. Of note, the American Heart Association proposed urinary AGT excretion as a diagnostic and prognostic biomarker of cardiorenal syndrome[49].

In summary, accumulating evidence supports the diagnostic biomarker value in the context of CKD of several urinary proteins and metabolites, with efforts to further prioritize them based on their potential clinical utility[7,96], in some cases, via systematic meta-analyses[7]. The value of markers that mainly reflect tubular damage (KIM-1, α1m, β2m, NAG and NGAL) has frequently been highlighted, with particular emphasis on the use of KIM-1, not only for detection of AKI but also in the context of incident CKD[96]. The independent associations of urinary EGF with incident CKD, in combination with assays that meet basic analytical precision requirements (<10% intra- and inter-assay coefficients of variation (CV)) also merit further investigation[97]. Importantly, inter-individual variance is high for KIM-1, as well as for NAG, NGAL and α1M (CVs ranging from 30% for KIM-1 to 71% for NGAL after normalization to urinary creatinine)[98]; high inter-individual variances (CV of 34%) have been noted for EGF[99]. This variability should be carefully delineated and considered when defining marker cut-offs[99]. In addition, both EGF and KIM-1 essentially reflect tubular alterations whereas CKD biomarkers should ideally reflect both tubular and glomerular alterations. Among novel multi-parametric biomarkers, the CKD273 peptide panel has been proven to allow early detection of DKD in a prospective clinical trial[83] and is now considered ready for clinical implementation[16]. The cost of this test is a potential drawback but a cost-effectiveness analysis in T2DM indicated that CKD273 was cost-effective compared with albuminuria, particularly in people at a high risk of DKD[100].

Prognostic markers

Several markers with prognostic potential have been reported in CKD (Table 2, detailed in Supplementary Table 2). In addition to its potential value as a diagnostic marker, urinary EGF has also been investigated in various aetiologies of CKD for its associations with the risk of progression, kidney failure and kidney-graft failure[3,66]. In T2DM, the urinary EGF-to-CCL2 ratio was independent of albuminuria and systolic blood pressure[101], which was similarly observed for urinary EGF-to-creatinine ratio in a subcohort of Chronic Renal Insufficiency Cohort (CRIC) study participants with diabetes[102]. The prognostic association of urinary EGF normalized to creatinine in the context of DKD progression was also supported by an assessment of the Veterans Affairs Diabetes in Nephropathy (VA NEPHRON-D) cohort, in which most participants (63%) had CKD stage 3[103] (Table 2). In addition, in postoperative AKI, individuals in the highest quartile of urinary EGF-to-creatinine ratio at 3 months post-discharge had a significantly lower risk of major adverse kidney events than those in the lowest quartile; a >2-fold increase in the urinary EGF-to-creatinine ratio compared with baseline at hospitalization pointed to a decreased risk of major adverse kidney events. Models combining urinary EGF-to-creatinine ratios at 3 months and clinical variables strongly discriminated for risk of kidney failure after 4 years (AUC = 0.96)[104]. The prognostic value of the EGF-to-creatinine ratio for progression of LN has also been demonstrated in a multi-ethnic cohort (Mexico and USA) of people with LN followed for at least 12 months[91]. Multivariate analysis supported associations of this ratio with time to doubling of serum creatinine.

In a 6-year follow-up Korean Cohort Study for Outcomes in Patients With Chronic Kidney Disease (KNOW-CKD) subgroups were stratified based on urinary AGT-to-creatinine quintiles for an assessment of associations with kidney outcomes[105]. Participants in the highest quintile were at an increased risk of deterioration of kidney function and kidney failure compared with those in the lowest quintile. A subgroup analysis indicated that this association was most substantial for individuals with BMI <23 kg/m^2 and UACR ≥300 mg/g. When T2DM or autosomal-dominant polycystic kidney disease was excluded in sensitivity analyses, the specificity of the association of AGT-to-creatinine ratio with kidney outcomes increased[105].

In a longitudinal study of people with CKD (CARE FOR HOMe cohort), urinary DKK3-to-creatinine levels >4,000 pg/mg at baseline were associated with a mean eGFR decline of 7.6% (−2.8 ml/min/1.73 m^2) over 12 months regardless of adjustment for confounders at baseline, including BMI, systolic blood pressure, diabetes, eGFR and albuminuria, compared with the reference group (DKK3-to-creatinine ≤200 pg/mg), where eGFR increased or did not change[106]. In groups of people with IgAN from the CARE FOR HOMe and STOP IgAN cohorts, the same study showed independent inverse associations of urinary DKK3-to-creatinine with changes in eGFR within 6 months. In addition, DKK3-to-creatinine ratio was also positively associated with kidney fibrosis, independently of aetiology, eGFR or albuminuria[106].

The prognostic value of urinary UMOD has been extensively investigated[35]. Low baseline UMOD in IgAN predicted rapid eGFR decline and correlated with degree of kidney fibrosis[107]. Similarly, increased UMOD was associated with a lower adjusted risk of eGFR decline in the prospective Cardiovascular Health Study[107] and the VA-Nephron D trial[103]. However, despite extensive studies, the specific biomarker value of UMOD has not yet been defined. Reasons underlying this uncertainty include the significant intra- and inter-individual concentration fluctuations and unexplained variability among populations.

Analysis of urine samples from the VA NEPHRON-D trial involving veterans with diabetes and albuminuria ≥300 mg/g indicated associations of the highest quartiles of the inflammatory markers CCL2 and CH3L1 with a higher risk of kidney function decline (defined as a decrease in eGFR ≥30 ml/min/1.73 m^2 if eGFR ≥60, ≥50% if eGFR <60 ml/min/1.73 m^2, or kidney failure) compared with the lowest quartile[108]. Associations with mortality were observed for urinary NGAL, IL-18, CCL2 and CH3L1 levels at baseline[108]. Similar associations of CCL2 and KIM-1 with kidney function deterioration (defined as incident kidney failure, or eGFR decline ≥40% from baseline) were also observed in a sub-cohort of CRIC participants with diabetes[102]. Accordingly, data from the Systolic Blood Pressure Intervention Trial (SPRINT)[57], showed that urinary levels of CCL2, CH3L1 and KIM-1 were positively associated with a composite outcome of 50% eGFR decline and kidney failure requiring dialysis or kidney transplantation. In the same cohort, IL-18 had a negative linear correlation with eGFR. However, analysis of samples from the CRIC study[109] suggested no independent associations of KIM-1 with CKD progression, the discrepancies between the studies being attributed to differences in the methods used for KIM-1 quantification.

CH3L1 and CCL2 have also been linked to the prognosis of CKD following AKI. In a large cohort of hospitalized people with and without AKI, levels of urinary CH3L1 and CCL2 were evaluated at 3 months post-discharge, with those in the highest marker quartiles experiencing the highest decline in eGFR[110]. The presence of AKI did not affect these associations. These markers were also associated with mortality and composite CKD outcomes (CKD incidence or progression, or kidney failure). To some extent, similar results were obtained when a broader panel of markers was measured at multiple time points during the first year post-discharge in the same cohort[111]. The most pronounced associations were observed for CCL2 and KIM-1, with each s.d. increase corresponding to a 2- to 3-fold increase in CKD risk.

Following a multi-parametric approach, in the context of IgAN, a combination of 237 peptides in a support vector machine-based classifier (IgAN237) was associated with eGFR slope and had significant added value to clinical parameters in discriminating IgAN progression[112]. In a prospective follow-up study, the panel was further validated for its independent value as a prognostic marker for eGFR decline[113].

A similar multi-parametric analysis combining protein and metabolite biomarkers into 'factors' was applied to individuals with diabetes and eGFR <60 ml/min/1.73 m^2 from two different cohorts (CRIC and REGARDS). Tubular function factors (represented by EGF, urine asymmetric dimethylarginine and urine symmetric dimethylarginine) were independently associated at baseline with eGFR decline in both cohorts. In addition, higher levels of tubular damage factors (represented by α1m, KIM-1 and CCL2) were independently associated with higher risk of CKD progression[114].

Large-scale metabolomic studies in urine, in some cases combined with serum analyses, have identified further markers associated with CKD progression[75,78]. C-glycosyltryptophan was associated with three main endpoints (kidney failure, combined end point of kidney failure and AKI and mortality) in the observational German Chronic Kidney Disease Cohort[115]. In a follow-up study[78], similar associations of the plasma levels of C-glycosyltryptophan were observed and further validated (in the case of the composite kidney end point) in an independent cohort (ARIC), but not the urinary associations. Large-scale metabolomic profiling combined with targeted metabolite quantification[116] in urine from people with diabetes at various CKD stages, revealed 13 metabolites representing amino-acid metabolism, including adenine, that were positively associated with a high risk of kidney failure. The prognostic potential of urinary adenine-to-creatinine ratio for kidney failure and all-cause mortality was further confirmed in a follow-up targeted large-scale multi-cohort analysis[117]. Subsequent multi-omics analyses of kidney-biopsy samples combined with animal studies specifically suggested a mechanistic role of adenine in DKD development, via activation of the mTOR pathway and subsequent enhancement of fibronectin production. In people with type 1 diabetes treated with empagliflozin, UAdCR decreased when comparing post- and pre-treatment adenine levels, raising the hypothesis that SGLT2 inhibition might inhibit the potential profibrotic effects of adenine[117].

In summary, multiple urinary biomarkers have frequently been investigated in the context of CKD progression in large-scale studies, sometimes with conflicting results, which might reflect differences in the populations under investigation, study power and/or analytical approaches. The independent associations of EGF and CCL2, individually measured and/or indexed to each other, with rapid eGFR decline in various cohorts[57,91,101,104,108,111], support further evaluation of their clinical utility. However, the abovementioned considerations (see 'Diagnostic markers' section) regarding the biological variability of these markers and the expected lack of specificity, particularly for CCL2, warrant caution. Results for DKK3, which is released by tubular cells in response to stress, have been consistent, justifying further advancement in the biomarker implementation pipeline[17,96]. Importantly, the links between DKK3 and WNT–β-catenin signalling, and the induction of fibrosis suggest potential as a predictive marker for targeted therapies[17].

Biomarkers for therapeutic monitoring

Large-scale studies on the potential of biomarkers to predict or assess therapeutic efficacy are scarce (Table 3, detailed in Supplementary Table 3). In a post hoc analysis of the CANVAS trial (the Canagliflozin Cardiovascular Assessment Study), levels of KIM-1 and CCL2 were evaluated in people with type 2 diabetes with CVD or CVD risk factors, and micro- or macroalbuminuria, at baseline and at week 52 of treatment with canagliflozin[118]. At the 52-week time point, decreased levels of CCL2 and KIM-1 (normalized to creatinine in both cases) compared with placebo were observed in the treatment group, with a concomitant reduction in UACR. Further modelling revealed that the relationship between SGLT2 treatment and KIM-1 was partly associated with the UACR and CCL2 reductions, linking the decrease in albuminuria to a reduction in inflammation (represented by CCL2) and a subsequent decrease in tubular damage (represented by KIM-1). The value of KIM-1 was further highlighted in a subsequent observational study involving people with CKD treated with dapagliflozin[119]. Urinary levels of KIM-1 but also of IL-1β and mtDNA copy number were significantly decreased at 6 months following SGLT2 treatment (a timepoint at which eGFR but not UACR changes could also be observed). In addition, KIM-1 and CCL2 levels were reduced in both low (<300 mg/g) and high (300 mg/g) albuminuria groups.

In a multi-cohort study, a peptide classifier was investigated for its potential to predict responses to angiotensin-converting enzyme inhibitor and angiotensin-receptor blocker treatment in people with diabetes[120]. Response assessment was based on the rate of progression (eGFR slope) and four different equations for eGFR estimation were used. Peptides differing consistently in abundance between progressors and non-progressors in the discovery set, regardless of the applied eGFR equation, were used for the development of a classifier, which consisted of 189 peptides (DKDp189). This classifier separated progressors from non-progressors in two validation cohorts with AUCs of 0.60 and 0.63, whereas significance in discriminating progressors was not reached in a subset analysis of individuals who had not received anti-hypertensive treatment.

In the context of an exploratory study, urine collagen fragment C3M was quantified in people with T2DM and moderate-to-pronounced CKD (eGFR ≥15 and <60 ml/min/1.73 m^2) treated with the glucagon-like peptide 1 receptor agonist (GLP-1RA) dulaglutide or insulin glargine[121]. Samples were collected for biomarker measurement at baseline, week 26 and week 52. After 1 year, dulaglutide mitigated eGFR decline and decreased UACR more than insulin glargine. C3M, a marker of type III collagen degradation, was higher in the dulaglutide group than in the insulin glargine group, correlating positively with eGFR at 52 weeks. This effect was more prominent in the macroalbuminuria subgroups. These findings suggested anti-fibrotic effects of dulaglutide linked to preservation of kidney function, meriting further investigation.

In summary, data related to monitoring and/or predictive biomarkers of therapeutic effect are scarce, lacking concrete associations with

patient outcomes. Targeted investigations evaluating factors directly linked to the mechanism of action of the drug could be a way forward. For example, the ongoing TOP-CKD randomized phase II trial is evaluating the effect of the anti-fibrotic drug pirfenidone over 12 months in people with CKD[122] by using a urinary biomarker associated with tubulo-interstitial fibrosis (N-terminal procollagen type 3 peptide (PIIINP)), as well as α1m and CCL2 or imaging changes compatible with kidney fibrosis on MRI, as primary outcome measures; changes in eGFR and UACR are secondary outcome measures.

Specific considerations for clinical implementation

Ideal biomarkers should reflect changes at the tissue level and be highly sensitive, specific (ideally differentiating aetiologies), easy to apply and affordable. As reviewed above, multiple associations with CKD and its evolution based on multi-cohort studies have been reported. However, translating these observations into reliable clinical applications is limited in most cases because the assays have not been approved for diagnostic purposes, have no clear definition of a cut-off value as required per disease context and/or have not been further validated in one or more prospective trials. Currently, two assays have been formally registered for diagnostic use: quantification of CKD273 by capillary electrophoresis-mass spectrometry (acknowledged by the US Food and Drug administration with a letter of support)[16], and quantification of urinary DKK3 by ELISA, certified for diagnostic use in humans in the EU[106] (according to the in vitro Diagnostic Medical Devices Regulation (EU) 2017/746). In addition, specific cut-off values have been proposed for these two assays (CKD273: 0.154 for early disease detection in individuals without albuminuria[123]; 0.343 for individuals with established CKD[124]; urinary DKK3: 4,000 pg/mg creatinine for people with a mean eGFR decline of 7.6% (95% confidence interval, decline of 4.3 to 10.8%) over 12 months[106] ; 354 pg/mg creatinine in people with heart failure to assess risk of eGFR decline at 1 year[125]); CKD273 has also been validated in a prospective trial[83]. Of the remaining markers that have been highlighted in this review, assays that met regulatory requirements are available for EGF, CCL2, KIM-1 and UMOD[97], and thus could be readily used towards establishing and/or validating proposed cut-offs (for example, for EGF in LN prognosis[91]).

However, multiple challenges undoubtedly arise in the case of CKD biomarkers. Associations between the biomarker and albuminuria are frequently observed and should be considered with caution when investigating biomarker potential, given that albuminuria is already of high diagnostic value, not only for CKD but also for other chronic diseases[126]. Another substantial confounder is the interference of CKD with diverse comorbidities. In addition to the well-known strong links between kidney diseases, and diabetes and CVD, kidney health has been associated with many other conditions that can independently affect urinary omic content, blurring the definition of biomarker specificity. In a large cohort study, increased urinary levels of KIM-1, CCL2 and α1 m were associated with frailty, whereas urinary β2m was associated with cognitive dysfunction independently of classical risk factors[127], akin to associations of albuminuria and eGFR with dementia and brain damage[128].

These observations further underscore the need for large-scale cohort studies to reach statistically robust outputs, which is the only way to define the added clinical value of a biomarker. Integration of data from independent studies is an important tool for increasing power. However, this approach is complicated by issues with data comparability, if different protocols for sample collection, assessment, eGFR calculation, definition of kidney function decline or endpoints are used, as reported in several meta-analyses on biomarkers.

The added value of using biomarkers in combination rather than individually, propelled by technological and methodological progress, has now been demonstrated sufficiently. This benefit might be attributed to the decreased effect of inter-individual variability. However, the clinical applicability and cost of such advanced multi-parametric approaches might limit implementation. Cost-effectiveness must be analysed in the broader context of the economic impact of each biomarker or panel, as the negative effect of higher marker cost might be compensated for or even overruled by higher specificity, sensitivity or earlier detection capacity compared with standard markers. Affordability in low-resource regions and among people with low incomes in high-resource countries must also be considered.

The health-economic balance might therefore differ geographically, depending on the local disease epidemiology and economy, and should be outweighed against current diagnostic, monitoring and prognostic standards (that is, albuminuria and eGFR). Coverage by public and private payers is variable and not always based on clear criteria, adding to the complexity[129–132]. Nevertheless, the role of technological evolution — increased availability of next-generation sequencing, mass spectrometry-based assays and high-density protein arrays — is evident and offers clear advantages by being granular enough to account for disease heterogeneity, with evidence of analytical and clinical validity in certain cases. In parallel, an evolution of regulatory guidelines is slowly occurring, reflecting a conceptual re-booting from simple single-molecule testing to more complex molecular profiling, as part of personalized disease-management plans[133]. Nevertheless, regulatory hurdles remain substantial, including the current approach to use serum creatinine or eGFR, and particularly albuminuria (all prone to confounding), as the standard with which new biomarker tests should be compared and show superiority. In addition, continuous changes in regulatory frameworks (for example, updated requirements for in vitro diagnostic medical devices in the EU described in Regulation (EU) 2017/746 from May 2022) can be further complicated by differences in national legislative interpretations and adaptations. Privacy regulations (such as General Data Protection Regulations) also complicate the use of large databases[134]. The need for health-economic evidence in addition to clearcut clinical data further contribute to this complexity, as well as the need to consider ever growing clinical and biological insights, and novel therapies and test methods.

In addition to cost–benefit, the impact of a biomarker in clarifying pathophysiology, accelerating drug development and enabling toxicity monitoring and therapeutic efficacy assessment should not be underestimated. These considerations are of particular importance in CKD as the gold standards (that is, albuminuria and eGFR) have minimal or no value in these contexts. Expansion of coordinated efforts such as those of the CKD Biomarkers Consortium to validate biomarkers for clinical implementation and adoption are highly useful in this context. A multidisciplinary approach to coordinating activities at all relevant levels, including basic research, clinical, regulatory and political institutions, as well as targeting awareness of novel approaches by information and education, is needed for biomarker implementation. Importantly, all of those aspects should be considered in the context of the urgent need to forestall inequities in access to good-quality health care[135,136]. Accordingly, reported approaches to health-economic modelling at an early phase of biomarker development[137,138] might be helpful in streamlining and providing an implementation focus in the current biomarker landscape.

Conclusions

Multiple studies highlight associations between urinary molecular content and kidney-tissue biology and histopathology, CKD phenotypes and disease progression. Powerful contemporary techniques allow the exploration of soluble urinary materials, cell debris and exosomes, improving understanding of disease pathophysiology and supporting biomarker development. Compared with soluble urinary materials, cell debris and exosomes are underexplored for biomarker purposes, owing to technical challenges associated with their extraction and the need for robust analytical protocols for their study. We propose that coupling exosomal findings with in vitro and in vivo functional studies is the optimal research option, as this approach promises to provide valuable information on communication between specific kidney compartments and the propagation of initial localized injury to the whole nephron.

Investigation of metabolites has uncovered novel research areas with insights into the gut–kidney axis; furthermore, coupling metabolomics to functional studies helped to identify uraemic toxins (reviewed in Glorieux et al.[68] and Rosner et al.[69]). Whereas information at the metabolome, DNA and RNA levels has mostly been explored for its biological relevance, protein profiles have been more successful in biomarker development. In addition to classical markers such as NGAL, KIM-1, CCL2, CH3L1, α1m and β2m, advanced technologies have identified other biomarker candidates and biomarker panels of prognostic and predictive potential, subsequent to multicentre investigations.

In conclusion, we are at an exciting crossroads in CKD diagnosis and management, with a growing armamentarium of powerful therapeutics and multiple biomarker candidates with demonstrated added value, in parallel with increasing availability of molecular information of an unprecedentedly high resolution[139]. The adoption of open-minded comprehensive approaches is urgently needed to harness this great potential towards both integrated and particularly implementation-focused, ethically sound research to the benefit of all people with kidney disease and their families.

Published online: 07 October 2025

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Acknowledgements
The authors are members of and would like to cordially thank the European Uraemic Toxin Group (EUTox) and the COST Action PerMediK, CA21165, supported by COST (European Cooperation in Science and Technology) for insightful discussions. We are also grateful to F. Paradeisi for the preparation of the submitted figure drafts.

Author contributions
All authors researched data for the article, made substantial contributions to discussions of the content and wrote, reviewed or edited the manuscript before submission.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41581-025-01008-2.

Peer review information *Nature Reviews Nephrology* thanks Jeffrey Schelling and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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