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Potential biomarkers of chronic kidney disease progression among kidney-derived proteins; a review

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ABSTRACT

The incidence and mortality rate of kidney disease and its progression to end-stage disease have predominantly increased worldwide. Other morbid conditions, such as diabetes and hypertension, are major risk factors for kidney disease. Detection of kidney disease is difficult due to its heterogeneity and complex pathophysiology. Kidney injury and advanced stages of the disease are currently assessed by traditional biomarkers such as serum creatinine, albuminuria, proteinuria and estimated glomerular filtration rate. Numerous biomarkers derived from the kidney involved in endothelial dysfunction, inflammatory processes and tubular cell damage are potential targets for disease progression management. The review summarized potential biomarkers of chronic kidney disease (CKD) to improve patient care in various clinical practices with an increased focus on loss of kidney function.

Review

Implication for health policy/practice/research/medical education:
Acquiring knowledge regarding potential noninvasive biomarkers of kidney-derived proteins in the progression of CKD may provide information that will aid in the development of novel therapeutics to improve the clinical outcomes and quality of life of individuals with kidney disease.

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Introduction

Kidneys are essential in maintaining the health and vitality of the human body and are responsible for homeostasis in blood filtration, excretion, fluid and electrolyte balance, controlling high blood pressure and the production of red blood cells. As per the World Health Organization (WHO), the estimated mortality rate due to kidney disease is 5-10 million, with 2.3-7.1 million due to end-stage kidney disease (1). Several secondary factors contribute to renal dysfunction, including hyperlipidemia, proteinuria, hypertension, exposure to nephrotoxins and chronic inflammation. Heterogeneous pathways such as hypoxia and oxidative stress contribute to renal damage, further irreversibly altering the structure and function of the kidney and leading to the development of chronic kidney disease (CKD) (Figure 1).

Chronic kidney disease is described as the gradual loss of kidney function. Over the past decades, the prevalence of CKD has increased more than 3-fold and has been reported to be approximately 11% in high-income countries. The people in the lowest socioeconomic quartile had a 60% higher risk of progressive CKD than those in the highest quartile. Risk factors for developing CKD differ between races and countries. CKD is characterized by increased and irreversible nephron loss, microvascular damage, reduced renal regenerative capacity, oxidative stress and inflammation (2). Decreased kidney function indicated by glomerular filtration rate (GFR) less than 60

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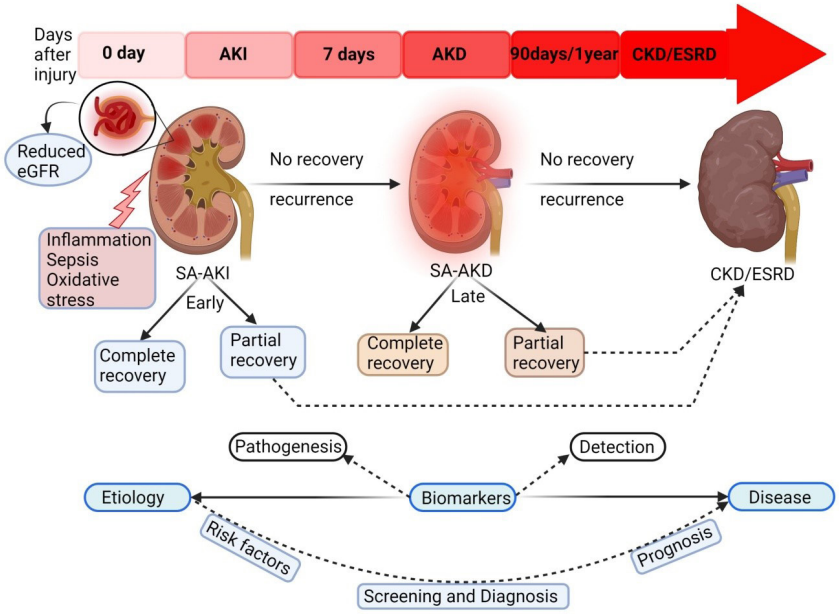

Figure 1. Range of outcomes following kidney injury.

mL/min/1.73m^2 regardless of underlying cause. Estimated GFR (eGFR), albuminuria and serum creatinine, which are conducted as conventional biomarkers, are elevated in the loss of kidney function and reach an advanced condition. Loss of the integrity of epithelial cells, tubular cell injury, accumulation of collagen (type I and III) and failed wound healing of kidney tissues lead to fibrosis, which is a major contributor to nephron loss/failure (2). Impaired activity of endogenous renal matrix-degrading proteases enhances interstitial matrix accumulation, but the specific pathways involved remain unclear. There is an urgent need for sensitive biomarkers that can detect disease earlier than traditional biomarkers and predict disease progression. In this review, we selected biomarkers of CKD involved in various pathophysiological mechanisms and summarized the current understanding of these biomarkers in renal disease progression (Table 1; Figure 2).

Biomarkers of glomerular function

Beta trace protein (BTP) is a prostaglandin D2 synthase that promotes the conversion of prostaglandin H2 to prostaglandin D2. It belongs to the protein family lipocalin, monomeric glycoprotein with a low molecular weight of 23-29 kDa. BTP is expressed in kidneys and is present in biological fluids such as blood and urine. In patients with CKD, an increased concentration of BTP in serum or plasma and urine significantly correlated with a decreased GFR. BTP is a promising novel endogenous biomarker of GFR in CKD. BTP is measured by nephelometric, immunodiffusion, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence in biological fluids. BTP converts arachidonic acid derivative prostaglandin H2 into more stable biologically active prostaglandins (PGs), including PGD2. Prostaglandins are produced in the kidney and have a role in triggering the inflammatory response, thereby contributing to the progression of kidney disease. PGD2 is involved in various physiological functions, such as sleep induction and regulation, nociception, nitric oxide (NO) release, inhibition of platelet aggregation, and inflammatory mediator modulation. BTP as noninvasive marker of impaired GFR. Urinary BTP has a sensitivity of 76.9% and a specificity of 80%, and serum BTP shows 77% sensitivity and 96.7% specificity and has higher utility in clinical use (3).

Beta-2-macroglobulin (B2 M) has a molecular mass of 11.8 kDa. The tertiary structure of B2 M is similar to that of immunoglobulins. B2 M is associated with major histocompatibility complex I/human leukocyte antigen I (HLA-I) and neonatal Fc receptor (FcRn) on the surface of all nucleated cells involved in albumin homeostasis, IgG recycling and iron metabolism. It can shed during membrane turnover and is thus be detected in various body fluids, including synovial, cerebrospinal, and saliva. B2 M passes through the glomerulus with a high sieving coefficient and is reabsorbed and catabolized by proximal tubules. B2 M concentration decreased by extra-renal factors such as glucocorticoids in a dose-dependent manner impairs the usefulness of B2M as a marker of GFR. B2 M is measured by turbidimetry, nephelometry and immune assays. The serum B2M concentration reflects inflammation, and increased urinary B2M indicates tubular dysfunction. Further studies are required to explore the role of B2M in oxidative stress. Urinary B2M has been clinically used for treating acute kidney injury due to sepsis. The interaction between B2 M and the alpha chain of HLA-I is crucial for antigen presentation. The role of FcRn provides a mechanistic link between B2M and albumin. FcRn expressed in the kidney (in podocytes,

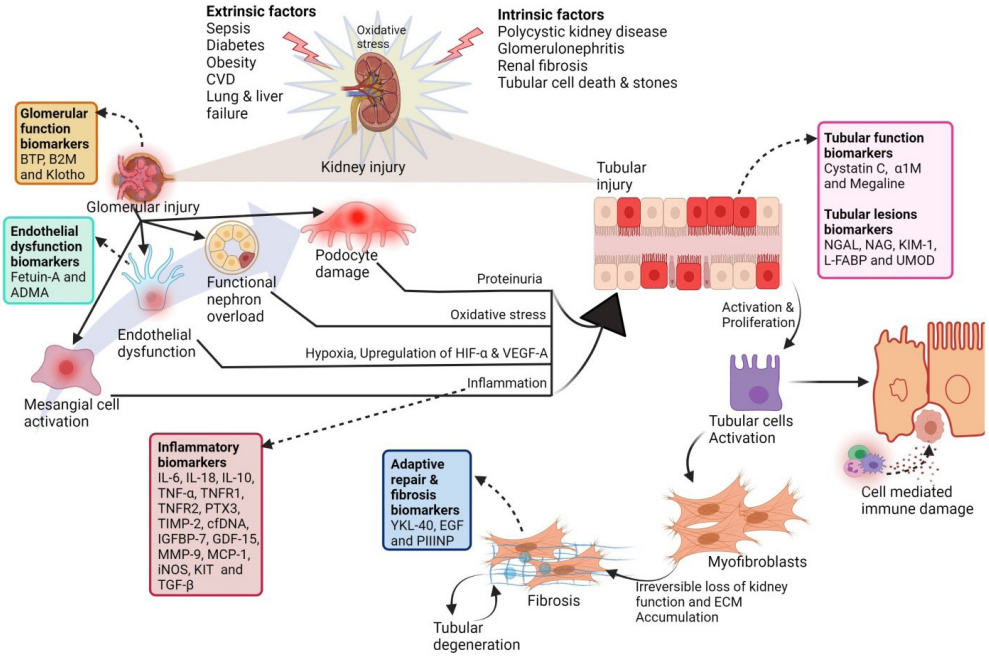

Figure 2. Schematic representation of involvement of potential biomarkers in chronic kidney disease

endothelial cells, and proximal tubular cells) facilitates albumin clearance. B2M containing FcRn triggers fibrosis through the p38MAPK pathway in the kidney. Inhibition of the FcRn/p38MAPK pathway reduces cell apoptosis and podocyte injury. The study by Dylewski et al on podocyte-specific FcRn knockout mice showed significantly reduced albuminuria and glomerulosclerosis compared to controls, suggesting that podocyte FcRn has a role in disease progression (4). Further investigations are required to confirm whether FcRn-mediated albumin absorption is a possible novel mechanism linking the activation of the renin–angiotensin system, oxidative stress, and the progression of kidney disease.

Additionally, klotho is a single-pass transmembrane protein with a wide spectrum of activity related to beta-glucuronidases. The kidney is a major organ that maintains soluble klotho homeostasis. Soluble klotho is detected in blood, cerebrospinal fluid and urine. The reduced levels of klotho observed in the disruption of calcium and phosphate metabolism, tissue injury, inflammation, oxidative stress, and fibrotic processes are positively correlated with the loss of kidney function. Klotho regulates NO production, thereby suppressing oxidative stress, anti-inflammatory activity, and synthesis of vitamin D and influencing fibroblast growth factor (FGF) signaling (5). Klotho is associated with improvements in mineral metabolism, attenuation of renal fibrosis and impedance of CKD progression. Immune assays are conducted for the quantification of klotho. A significant decrease in serum klotho levels with increased albumin excretion was observed during renal injury. Klotho serves as a co-receptor of FGF23 and promotes phosphaturia in the regulation of phosphate metabolism and interrupts intestinal phosphate absorption by inhibiting 1α,25-dihydroxyvitamin D3. In the proximal renal tubule, the FGFR-klotho complex activates various signaling pathways, such as serum/glucocorticoid-regulated kinase (SGK)-1, and extracellular signal-regulating kinase-1/2 activates SGK-1, which later phosphorylates the Na+/H+ exchange regulatory cofactor, thereby downregulating sodium phosphate cotransporter and leading to increased urinary phosphate excretion. Hyperphosphatemia is a principal regulator of FGF23 secretion during CKD. Increased levels of FGF23 and decreased levels of klotho are correlated with eGFR in CKD. The novel link between the FGF-klotho complex and dysregulated ion metabolism has important pathophysiological implications and may act as a therapeutic target for CKD.

Biomarkers of tubular function

Cysteine protease inhibitor (CysC) is a nonglycosylated low molecular mass 13.3-kDa protein that acts as a protease inhibitor produced from all nucleated cells and is detected in all body fluids. The kidney is a catabolic site of CysC that passes through the glomerulus with a sieving coefficient of 0.84 (6). CysC levels are correlated with eGFR. Filtered CysC is reabsorbed in the proximal tubule by megalin–cubilin receptor-mediated endocytosis. A meta-analysis including 19 studies concluded that serum CysC is a potential biomarker showing a sensitivity of 85% and specificity of 87% for detecting mild reductions in GFR in CKD (7). The proposed CKD-epidemiology collaboration (CKD-EPI) equation using CysC concentration improves the predictive performance of eGFR. Large prospective studies are needed to confirm the clinical implications of CysC in CKD management and if there are any associated

Table 1. List of biomarkers of chronic kidney disease

Molecule	Molecular mass in kDa	Source	Biological function
Glomerular function biomarkers			
Beta trace protein	23	Cerebrospinal fluid	Inhibition of platelet aggregation, vasodilatation, and recruitment of inflammatory cells
β2-microglobulin	11.8	All nucleated cells	Regulation of immune response
Klotho	130	Kidneys: analyzed in urine	Anti-ageing, preventing excess positive phosphate balance, calcium ion homeostasis
Tubular function biomarkers			
Cystatin C	13.3	All nucleated cells	Cysteine-type endopeptidase inhibitor activity
Alpha -1-microglobulin	26	Liver	Human radical scavenger and antioxidant
Megalin	600	Podocytes and renal proximal tubular cells	Regulation of renin-angiotensin system
Tubular lesions biomarkers			
Neutrophil gelatinase associated lipocalin	25	Renal tubular cells	Antimicrobial humoral response and cellular iron ion homeostasis
N acetyl beta-D-glucosaminidase	140	Renal proximal tubular cell	Early indication of tubular dysfunction resulting from nephrotoxic damage
Kidney injury molecule-1	104	The proximal tubule of the kidney	Recognizes apoptotic cells directing them to lysosomes
Liver-type fatty acid binding protein	14	Kidney: proximal tubular cells and lysosomes	Antioxidant activity
Uromodulin	105	Kidneys	Calcium ion binding and cellular defense response
Endothelial dysfunction biomarkers			
Fetuin-A	60	Liver	Cysteine-type endopeptidase inhibitor activity, regulation of inflammatory response and calcified matrix metabolism
Inflammatory biomarkers			
IL-6	25	T-lymphocytes, monocytes, macrophages and endothelial cells	Regulation of neuroinflammatory response
IL-18	18	Monocytes, macrophages, dendritic cells, epithelial cells and keratinocytes	Regulation of cell adhesion, signaling receptor activity and Th1 and NK cell activation
IL-10	20.5	Innate immune cells	Anti-inflammatory properties
TNF- alpha	52	Macrophages, lymphoid cells and renal parenchymal cells	Cytokine activity, regulation of neuroinflammatory response and TNF-mediated signaling pathway positive regulation of phagocytosis
Pentraxin-3	40	Stromal and myeloid cells	Positive regulation of phagocytosis
Tissue inhibitor of metalloproteinases-2	24	Most of the cell types	Negative regulation of mitotic cell cycle and Ras protein signal transduction and neutrophil degranulation
Insulin-like growth factor-binding protein-7	29	Most of the cell types	Cellular response to hormone stimulus
Growth/differentiation factor-15	34	Late-stage erythroid precursors in the bone marrow	Growth factor activity and Activation of MAPK activity
Transforming growth factor-β	44	White blood cell lineages	Induce production of extracellular matrix and angiogenesis-inducing factor
Adaptive repair and fibrosis biomarkers			
YKL-40	42.6	Smooth muscle cells	Activation of NF-κB-inducing kinase activity
Procollagen type III N-terminal propeptide	44	Released during Synthesis of new type III collagen	Assessing renal function

comorbidities. CysC has an ROC of 91.7% diagnostic accuracy for glomerular lesions in preeclamptic women (7). CysC is measured by nephelometric and automated immunoassays. Cysteine protease inhibitor increases extracellular matrix production and collagen breakdown (i.e., fibroblasts and mesangial cells). CysC expression is upregulated by pro-inflammatory lipopolysaccharides (LPS) and interferon γ in monocyte-derived dendritic cells, and TGF-β alters CysC levels associated with connective tissue remodeling during the immune response after kidney injury. CysC regulates the phagocytic function of polymorphonuclear neutrophils.

Megalin is a single transmembrane receptor protein belonging to the low-density lipoprotein receptor (LDLR) superfamily with a molecular weight of 600 kDa. The structure of megalin resembles LDLR-related protein 1 (LRP1); hence, it is known as LRP2. It is expressed in the proximal tubule and podocytes. The large extracellular domain and transmembrane region allow megalin to bind with more than 50 ligands. Ligands of megalin are insulin, insulin-like growth factor, lipoproteins, albumin, retinol-binding protein, transcobalamin-B12, EGF, lysozyme, cytochrome c, alpha-amylase, prolactin, CysC, liver-type fatty acid-binding protein (L-FABP), alpha 1 and beta 2-microglobulin (8). Angiotensinogen (Agt) is one of the ligands of megalin, and reabsorption of Agt contributes to the production of intrarenal angiotensin II (Ang II), which further activates sodium-hydrogen antiporter-3 and epithelial-sodium channels, thereby leading to sodium retention and edema formation. Reabsorption of Agt by megalin increases intrarenal Ang II in podocyte injury. Megalin is an endocytic receptor for prorenin in the renin-angiotensin system and a determinant of urinary renin levels. Urinary excretion of megalin is associated with renal oxidative stress in patients with CKD. Increased albumin induces megalin-dependent tubulointerstitial inflammation and fibrosis, contributing to CKD progression. Modulation of megalin function is a new approach for monitoring kidney injury. Studies on the regulatory expression of mesangial megalin would provide informative insight on its potential involvement in renal disease.

Alpha-1-microglobulin (A1 M) is a heme-binder glycoprotein with a molecular weight of 26 kDa. A1 M is part of the lipocalin protein family and is synthesized in the liver. A1 M has a role in RBC development and stability; therefore, it has a therapeutic approach in erythropoietic abnormalities. It is a physiological antioxidant and radical scavenger that protects cells and tissues against oxidative damage by activating the nuclear factor erythroid 2-related factor (Nrf2) cytoprotective pathway. Nuclear factor erythroid 2-related factor is a transcription factor regulating the expression of antioxidants in response to oxidative damage in tubular epithelial cells. The Nrf2 pathway promotes endogenous cellular A1 M and *HMOX-1* gene expression (9). Higher levels of A1 M were observed during kidney injury. Urinary-A1 M shows a sensitivity of 82% and specificity of 69% in hemorrhagic fever with renal syndrome patients (10). Urinary A1M is measured by immunonephelometric assay, and the levels are associated with faster CKD progression and higher mortality.

Biomarkers of tubular lesions

Neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein with a molecular weight of 25 kDa that belongs to the lipocalin family. It is identified in neutrophils and stored in granules of mature neutrophils. NGAL is a ubiquitous, iron-carrying protein also known as lipocalin 2 (LCN2) that binds to matrix metalloproteinase-9 in human neutrophils. NGAL is predominantly expressed in tubular epithelial cells during infection, intoxication, inflammation and ischemia. During sepsis, NGAL is a sensitive gene to tubular cell damage through toll-like receptor 4 (TLR4) and the nuclear factor kappa-light-chain-enhancer of activated B (NF-kB) pathway (11). NGAL promotes the expression of CD11c and M1 macrophages as an anti-inflammatory mediator of the NF-kB-STAT3 loop. NGAL is measured by western blot and ELISA. Urinary NGAL levels were proportional to interstitial fibrosis and tubular atrophy but inversely correlated with eGFR in CKD. Serum NGAL showed a sensitivity of 80% and specificity of 82% and was a significant predictor of CKD progression (12). In particular, NGAL is a novel potential early biomarker of kidney injury, and further studies are needed to confirm the advantages of its diagnostic value in clinical approaches. NGAL is stable in urine up to 48 hours at 2-8°C. Multi-centric prospective studies are required to validate NGAL in disease progression.

N acetyl beta-D-glucosaminidase (NAG) is a glycolytic enzyme with a molecular weight of 140- kDa that is expressed in renal proximal tubular cells. Levels of urinary NAG are directly associated with renal dysfunction. Urine NAG levels had an AUC of 0.81, which is a better predictive value than eGFR (AUC = 0.74) (13). The detection of urine NAG is simple and noninvasive and is a very sensitive and reliable indicator for determining renal tubular function in various conditions, such as injury or dysfunction due to inflammation, hypoxia, nephrotoxic drugs and diabetes. N acetyl beta-D-glucosaminidase inhibits LPS-induced cytokine formation, whereas LPS is an inflammatory agent activated by the TLR4-NFκB pathway, leading to the expression of inflammatory cytokines in macrophages. Urinary NAG is used as an early marker in the diagnosis of diabetic nephropathy.

Kidney injury molecule-1 (KIM-1) is a type 1-cell membrane glycoprotein with a molecular weight of 104 kDa that consists of a 90 kDa soluble and 14-kDa membrane-bound fragment. It is also known as T-cell immunoglobulin and mucin domain-containing protein-1 (TIM-1). KIM-1-1 is expressed significantly

in proximal tubular cells following ischemic injury due to the extracellular domain of matrix metalloproteinase. KIM-1 acts as a phosphatidylserine receptor that mediates phagocytosis of apoptotic bodies and cell debris during kidney recovery and tubular regeneration. KIM-1-1 modulates translational changes by interacting with phosphatidylinositol-3 kinase (PI3K) in proximal tubular cells. Elevated levels of KIM-1 are correlated with inflammation and fibrosis in clinical kidney damage. Elevated levels of urinary KIM-1 are observed in the early stages of CKD (G1 & G2) and are associated with an increased urine albumin-to-creatinine ratio. Elevated KIM-1 levels correlated with decreased eGFR, suggesting a predictor of renal function deterioration in healthy middle-aged patients. Continued expression of KIM-1 provides a link between acute and recurrent kidney injury with progressive CKD. KIM-1 levels assessed by lateral flow dipstick. The combination of KIM-1 and interleukin 18 (IL-18) resulted in improved identification of higher risk patients. Urinary KIM-1 showed a sensitivity of 74% and specificity of 86% during kidney injury (14). KIM-1-1 is a hallmark of proteinuric, toxic and ischemic kidney damage. Urinary KIM-1 has clinical application in the identification of drug-induced proximal tubular injury. More studies are needed on anti-TIM/KIM-1 antibodies in preventing renal disease.

Liver-type fatty acid binding protein (L-FABP) is a 14-kDa protein belonging to a large superfamily of lipid-binding proteins selectively binding to free fatty acids. Cytoplasmic FABP is a cytoplasmic protein expressed in a variety of tissues. There are two types of FABP; liver-type (L-)FABP and heart-type (H-)FABP produced in the renal proximal tubule and renal distal tubule, respectively. Liver-type fatty acid binding protein protects cells from oxidative stress induced by H_2O_2 via peroxisome proliferator activated receptor-α (PPAR-α), which increases L-FABP expression. Li et al studied mice with IgA nephropathy and determined that it reduced oxidative stress by regulating the expression levels of PPAR-α and L-FABP (15). Hypoxia is the main inducible factor in the expression of the L-FABP gene containing the hypoxia-inducible Factor 1α response element. Increased expression levels of renal L-FABP and high urinary excretion observed in various stressors, such as hyperglycemia, urinary protein, toxins, tubular ischemia and salt-sensitive hypertension, correlated with the severity of tubulointerstitial injury. Renal hL-FABP significantly suppresses the macrophage chemotactic and activating factors MCP-1 and MCP-3 and reduces the deposition of type IV collagen. A study estimated L-FABP during kidney injury, showing a sensitivity of 74% and specificity of 82% (16).

Uromodulin (Umod) is also known as a Tamm-Horsfall protein. It is a glycosylphosphatidylinositol-linked glycoprotein with a molecular weight of 105 kDa. It is a kidney-specific protein exclusively synthesized from renal tubular epithelial cells and abundantly present in urine. Umod is an isolated glycosylated mucoprotein with a large number of cysteine residues. It is involved in the regulation of salt homeostasis and is involved in immunologic renal protection, such as defending against urinary tract bacterial infections and inhibiting nephrolithiasis. Urinary Umod is involved in the regulation of tubular Na+/K+/Cl− transport and exhibits immunomodulatory properties. Decreased levels of serum Umod are associated with kidney function and the structural integrity of renal parenchymal/tubular cells. Serum Umod is a kidney-specific biomarker reflecting the integrity and functional viability of the thick ascending limb of the loop of Henle. Increased serum Umod is independently associated with the progression of CKD. Serum Umod showed a sensitivity of 57% and specificity of 100% (17). Well-known genome-wide association studies (GWASs) in humans have identified common variants in the Umod gene as risk factors for CKD. Single nucleotide polymorphisms in the promoter region of the Umod gene are associated with a decline in the occurrence of CKD. A trans-ancestry GWAS meta-analysis in CKD genetics identified 147 loci that were likely to be applicable in association with alternative independent markers of kidney function, resulting in molecular targets for potential therapeutics to improve CKD treatment (18).

Biomarkers of endothelial dysfunction

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthases. ADMA is an analog of l-arginine and most commonly occurs in human circulation. Some physiological factors, such as endothelial damage, cell senescence, glomerular hypertension and salt accumulation, lead to increased levels of ADMA, predicting the development of renal damage and the risk of cardiovascular disease among patients with end-stage renal disease. ADMA is a novel biomolecule that serves as a biomarker in CKD predicting eGFR<60 mL/min/1.73 m^2 with a sensitivity of 87% and specificity of 83% (19). Increased ADMA levels inhibit the synthesis of NO, thereby damaging endothelial function and stimulating renal impairment.

Fetuin-A is a multifactorial glycoprotein that is considered the most powerful circulating inhibitor of hydroxyapatite formation and is also named the alpha2-heremans-schmid glycoprotein. Fetuin-A is an anti-inflammatory protein with a molecular weight of 52 kDa and is a member of the cystatin superfamily. It is predominantly synthesized in hepatocytes and secreted into the bloodstream, attenuating the inflammatory response. The serum fetuin-A concentration predicting mortality in dialysis. The levels of serum fetuin-A are considerably lower in DKD. Serum fetuin-A levels are associated with proinflammatory cytokines such as IL-6, IL-18 and tumor necrosis factor alpha (TNF-α). Fetuin-A is involved in pathogenesis and provides new insight into the etiology of kidney disease.

Biomarkers of inflammatory response

Inflammation is a key risk factor of kidney damage. Activated leukocytes and proinflammatory cytokines are involved in tubular cell injury by activating the NF-κB and MAPK signaling pathways by upregulating IL-6, IL-10, IL-18 and TNF-α. Proinflammatory cytokines are produced when renal glomeruli, tubular epithelial cells and immune cells undergo stress conditions. Increased inflammation causes more pronounced collagen I deposition, and higher expression of α-smooth muscle actin, fibroblast-specific protein-1, fibronectin, vimentin and MMP-2 develops renal fibrosis. Inflammatory processes are activated in early stages of CKD and drive impairment of renal function.

Furthermore, IL-6 is a secreted glycoprotein with a molecular weight of 25 kDa. It regulates innate and adaptive immune responses and is a major target for clinical intervention in proinflammatory conditions. Although not all IL-6-associated diseases respond to IL-6 blockade, a better understanding of the underlying mechanisms of IL-6 pathways will help to identify the best treatment for IL-6-associated diseases in the future. IL-6 trans-signaling is critical in regulating the transition from neutrophils to monocytes, thereby preventing tissue damage. Targeting IL-6 trans-signaling by Fc-gp130 is a promising new therapeutic strategy to treat renal fibrosis.

Likewise, IL-10 is an anti-inflammatory cytokine produced by various cell types, including regulatory T cells. It inhibits proinflammatory cytokines such as IL-1, TNF-alpha, IL-6 and IL-18. Li et al demonstrated that after the onset of unilateral ureteral obstruction, more severe inflammation and fibrosis development occurred in the kidneys of mice lacking IL-10 than in the kidneys of wild-type mice. IL-10 knockout mice upregulate inflammatory cytokines and chemokines, including RANTES, monocyte chemoattractant protein-1, IL-6, IL-8 and TNF-α, in the kidney (20). Local immunotherapy with IL-10 in hyaluronic acid hydrogels reduced macrophage infiltration, the number of apoptotic cells and the size of fibrosis, confirming the potential use of IL-10-containing hydrogels in treating renal fibrosis. IL-10 suppressed the production of proinflammatory cytokines, thereby reducing renal function.

IL-18 is a member of the IL-1 family known as interferon-gamma inducing factor with a molecular weight of 18 kDa. Serum and urinary levels of IL-18 are elevated in diabetic nephropathy and independently correlated with urinary albumin excretion. Augmenting the action of the Nrf2 signaling pathway and its downstream mediators in CKD has the potential to attenuate, arrest, or even reverse the decline in kidney function.

Tumor necrosis factor-alpha is a proinflammatory cytokine responsible for various cell signaling events in necrosis and cell apoptosis. TNF-α has a role in the maintenance and homeostasis of the immune system, inflammation and host defense by resisting infection. TNF-α binds to cell membrane receptors, and either TNFR-1 or TNFR-2 exerts an effect on cells. In recent decades, monoclonal antibodies and an extracellular portion of human TNF-α receptors have been explored in renal fibrosis. However, the risk of an immunological response and undesired side effects suggest the need to develop more effective therapies to control TNF-α level. A study in an animal model found that macrophage-specific deficiency of Krüppel-like factor 4 (KLF4), a zinc-finger transcription factor, augmented M1 polarization and the expression of TNF-*α* (KLF4's downstream effector) in macrophages infiltrating the kidney as well as exacerbated glomerular matrix deposition, tubular damage, and interstitial fibrosis. Mice with macrophage-specific TNF-alpha deletion exhibited decreased kidney damage and fibrosis. Macrophage KLF4, a DNA-binding transcriptional regulator, ameliorates CKD by mitigating TNF-dependent injury and fibrosis.

Pentraxin 3 (PTX3) is a conserved plasma protein with a molecular weight of 42 kDa that belongs to the pentraxin superfamily. PTX3 acts as a chemoattractant factor in various inflammatory cells. It responds to various stimuli, such as IL-1, IL-1β, TNF-α and LPS. Lower levels of PTX3 lead to decreased infiltration of macrophages, directly affecting endothelial cell P-selectin and altering molecular physiology. PTX3 and adropin reported elevated serum levels of PTX3 and significantly declined adropin in varying degrees of pathogenesis in diabetic kidney disease. Higher PTX3 levels are associated with reduced eGFR and independently predict the incidence of renal fibrosis. Circulating PTX3 appears to be a promising biomarker of kidney damage prior to the development of overt CKD. PTX3 has a sensitivity of 92.5% and specificity of 70% (21). Studies are needed to elucidate the pathophysiological mechanisms and to determine the clinical relevance of PTX3 in inflammation and endothelial dysfunction indicating nephron loss.

Tissue inhibitor of metalloproteinases-2 (TIMP2) is a 21-kDa protein that inhibits metalloproteinase activity endogenously, and insulin-like growth factor-binding protein-7 (IGFBP7) is a 29 kDa secreted protein that inhibits signaling through IGF-1 receptors. Under conditions such as cellular stress, ischemic, or septic kidney injury, renal tubular cells synthesize and release TIMP2-IGFBP7 (11). The sources of TIMP2-IGFBP7 and their pathophysiological roles have yet to be described. The diagnostic value of TIMP2-IGFBP7 as a novel biomarker for predicting kidney injury was validated and approved by the FDA for clinical application. TIMP2-IGFBP7 levels indicate the best accuracy and stability in patients with CKD by regulating cell cycle arrest. They are important mediators during renal epithelial cells undergoing G1 cell cycle arrest during conditions such as ischemic or septic kidney injury. TIMP2 stimulates p27 expression, and IGFBP7 increases the expression of p53 and p21. These p proteins block the effect of cyclin-dependent protein

kinase complexes (CyclD-CDK4 and CyclE-CDK2). More investigations, such as clinical trials, are needed to support the hypothesis that early recognition of kidney injury with TIMP2-IGFBP7 prevents further progression of disease, and the cost–benefit of the TIMP2-IGFBP7 test to the patient is unknown. Studies regarding the applicability of TIMP2-IGFBP7 and their pathophysiology are major priorities in various clinical contexts, and different disease spectra in diverse patient populations are needed to achieve improved outcomes. Adler et al concluded that urinary TIMP2-IGFBP7 is a reliable predictor of kidney injury with a sensitivity of 96.8% and specificity of 94.1% (22).

Growth/differentiation factor-15 (GDF-15) is a cytokine induced in response to ischemia and neurohormones. GDF-15 expression significantly and specifically reflects the pathophysiology of CKD progression. Increased levels of GDF-15 help to identify CKD patients with the highest risk for loss of kidney function. GDF-15 showed a sensitivity of 73% and specificity of 61% among CKD patients (23). Further studies are required to understand the signaling pathways and molecular mechanisms involved in the elevation of GDF-15 in CKD progression, which would be novel therapeutics and an emerging predictor of adverse clinical outcomes.

Cell-free DNA is present in various body fluids, such as blood, urine, synovial fluid, and cerebrospinal fluid. It refers to fragmented DNA mainly originating from blood cells during oxidative stress and inflammation causing DNA damage. Cell-free DNA derived from apoptosis, necrosis and DNA damage correlated with an enhanced inflammatory response in CKD. Elevated levels of cfDNA were predominantly observed in patients with DKD. Elevated circulating cell-free DNA (cfDNA) concentrations were independently associated with DKD. Urinary cf-mtDNA and cf-nDNA predict renal outcome in CKD patients. The levels of plasma cf-nDNA and plasma NGAL significantly correlated with disease progression. Hence, serum cfDNA is a good predictor of disease progression. The mechanism involved in the elevation of cfDNA in disease progression has yet to be defined. Further studies on genetic analysis are required to identify different types of DNA damage that amplify cfDNA during kidney injury.

Transforming growth factor-beta (TGF-β) is a multifactorial cytokine and a key regulator of various cellular processes, such as cell growth and differentiation, apoptosis, tissue repair and fibrosis pathogenesis. The factors involved in the activation of latent TGF-β include proteases, integrins, metalloproteinases and plasmin that undergo TGF-β/smad signaling. TGF-β is generally considered a profibrotic mediator during excessive extracellular matrix (ECM) production. Elevated levels of TGF-β are highly correlated with the production of ECM leading to CKD. Epigenetic modifications of DNA and histone proteins in TGF-β1/smad signaling have been identified in CKD. Latent TGF-β1 overexpression restricts fibrosis and inflammation during kidney disease. TGF-β is the main target for developing novel therapeutics to halt the most damaging stage in CKD.

Biomarkers of adaptive repair and fibrosis

YKL-40 is a 40-kDa inflammatory glycoprotein produced by neutrophils involved in modulating favorable responses during cellular damage. It is also known as cartilage glycoprotein 39 or chitinase-3-like protein 1. YKL-40 is produced by macrophages. Recurrence of cell injury and inflammation eventually leads to fibrosis. YKL-40 plays an important role in macrophage differentiation, proliferation of chondrocytes and fibroblast migration, remodeling of the extracellular matrix and reorganization of vascular endothelial cells. A study revealed that patients who underwent peritoneal dialysis and hemodialysis had elevated levels of YKL-40 when compared to healthy individuals. YKL-40 is a predictor of diabetic nephropathy with a sensitivity of 83% and specificity of 85% (24). Elevated levels of YKL-40 measured by ELISA in patients with different stages of CKD.

Procollagen type III N-terminal propeptide (PIIINP) is an amino-terminal propeptide of type III procollagen with a molecular weight of 44 kDa. When kidneys are affected by continued and progressive injury, they exhibit increased collagen production. PIIINP is released into urine and blood from the extracellular matrix. Individuals with higher PIIINP levels in their urine are at greater risk for CKD progression independent of albuminuria. Urine PIIINP is considered a noninvasive method associated with the incidence of renal fibrosis. Urine PIIINP showed a sensitivity of 94% and specificity of 55% in CKD progression (25).

Challenges and future perspectives

The current dependence on only conventional biomarkers, such as creatinine and eGFR, may lead to longer treatment times. Molecular-level omics science has opened challenges in patient care. The identification of potentially new, sensitive and identified biomarkers from experimental studies has greater potential to detect early renal disease than traditional markers. In addition, large prospective studies are needed to confirm the clinical applicability of existing biomarkers. Molecular phenotyping based on distinct disease signatures may lead to personalized treatment for kidney disease. Ongoing research is mainly working to halt disease progression by investigating circulating microRNAs and omics profiling. Constructing a kidney tissue-based atlas to describe disease with corresponding plasma and urine biomarkers further allows assessment in comparison with the true gold standard method of kidney biopsies and detection of novel biomarkers. Further studies are needed to describe biological pathways involved in kidney repair and long-term survival, which will help to develop novel therapeutics

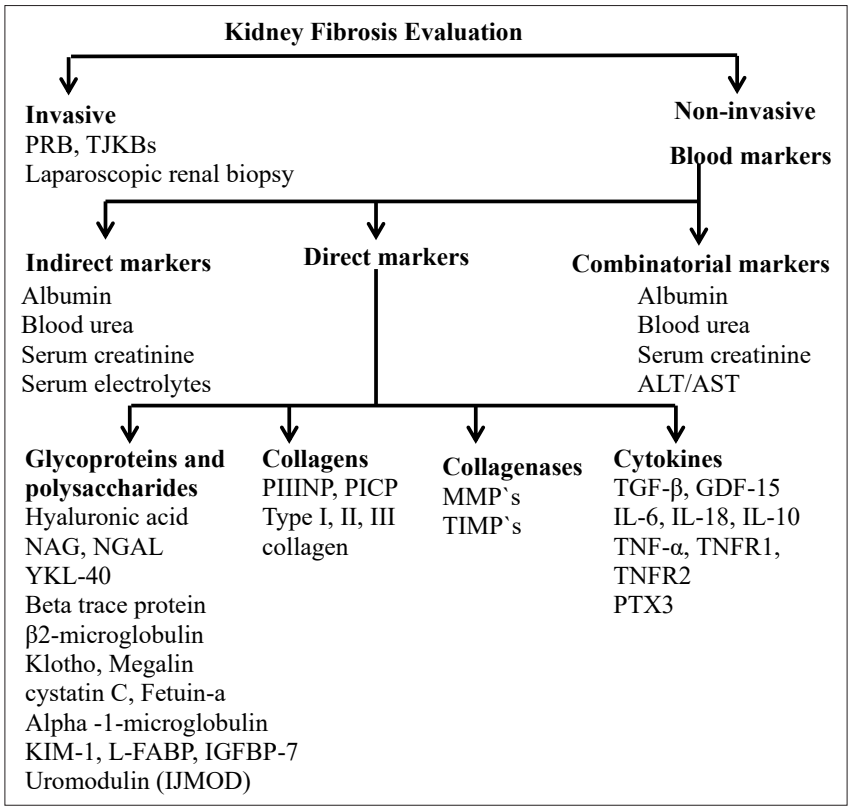

Figure 3. Algorithm of chronic kidney disease markers.

in nephrology. There is an emerging need for the discovery of specific, novel and reliable early biomarkers of disease progression and risk of morbidity. Studies on animal models are needed to capture the complexities of other overlapping diseases, such as diabetes, CVD/hypertension and aging. Currently, advancements in omics (genomics, transcriptomics and proteomics), CRISPR/Cas9 genome editing and bioinformatics tools help in identifying single or multiple biomarkers in one platform. Assessment of multiple biomarkers instead of a single biomarker is a reasonable approach to detect injury and to predict disease progression.

Conclusion

Numerous biomarkers are involved in the pathophysiological mechanisms of kidney damage with the potential to improve clinical management (Figure 3). These biomarkers help to detect kidney injury, locate cell damage and predict disease severity and its progression with associated morbidities. Even though markers are more sensitive to diseases, they were showing less specificity (i.e., B2 M, A1 M and GDF-15). Studying a combination of markers helps to identify disease progression, such as CysC, with the CKD-EPI equation. Future studies are underway to find more sensitive and disease-specific markers and to characterize the pathophysiological pathways involved in the kidney damage repair mechanism leading to long-term survival, which may provide new ways to develop novel therapeutics in CKD progression.

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Authors' contribution

SPK and PN: conceptualization, resources, and writing-original draft preparation.
VBB and SK: writing—review and editing.
SR: conceptualization, writing—review and editing, supervision.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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References

1. Luyckx VA, Tonelli M, Stanifer JW. The global burden of kidney disease and the sustainable development goals. Bull World Health Organ. 2018;96:414-422D. doi: 10.2471/BLT.17.206441.
2. Ruiz-Ortega M, Rayego-Mateos S, Lamas S, Ortiz A. Targeting the progression of chronic kidney disease.

2020;16:269-88. doi: 10.1038/s41581-019-0248-y.
3. Donadio C, Bozzoli L. Urinary β-trace protein: A unique biomarker to screen early glomerular filtration rate impairment. Medicine (Baltimore). 2016;95:e5553. doi: 10.1097/md.0000000000005553.
4. Dylewski JF, Tonsawan P, Garcia G, Lewis L, Blaine J. Podocyte-specific knockout of the neonatal Fc receptor (FcRn) results in differential protection depending on the model of glomerulonephritis. PLoS One. 2020;15:e0230401. doi: 10.1371/journal.pone.0230401.
5. Baranowska B, Kochanowski J. The metabolic, neuroprotective cardioprotective and antitumor effects of the Klotho protein. Neuro Endocrinol Lett. 2020;41:69-75.
6. Mussap M, Plebani M. Biochemistry and clinical role of human cystatin C. Crit Rev Clin Lab Sci. 2004;41:467-550. doi: 10.1080/10408360490504934.
7. Gomes H, Cabral ACV, Andrade SP, Leite HV, Teixeira PG, Campos PP, et al. Cystatin C as an indicator of renal damage in pre-eclampsia. Hypertens Pregnancy. 2020;39:308-13. doi: 10.1080/10641955.2020.1766488.
8. Mahadevappa R, Nielsen R, Christensen EI, Birn H. Megalin in acute kidney injury: foe and friend. Am J Physiol Renal Physiol. 2014;306:F147-54. doi: 10.1152/ajprenal.00378.2013.
9. Kristiansson A, Davidsson S, Johansson ME, Piel S, Elmér E, Hansson MJ, et al. α1-Microglobulin (A1M) Protects Human Proximal Tubule Epithelial Cells from Heme-Induced Damage In Vitro. Int J Mol Sci. 2020;21(16):5825. doi: 10.3390/ijms21165825.
10. Hansson M, Gustafsson R, Jacquet C. Cystatin C and α-1-microglobulin predict severe acute kidney injury in patients with hemorrhagic fever with renal syndrome. Pathogens. 2020;9:666. doi: 10.3390/pathogens9080666.
11. Schrezenmeier EV, Barasch J, Budde K, Westhoff T, Schmidt-Ott KM. Biomarkers in acute kidney injury - pathophysiological basis and clinical performance. Acta Physiol (Oxf). 2017;219:554-72. doi: 10.1111/apha.12764.
12. Sun IO, Shin SH, Cho AY, Yoon HJ, Chang MY, Lee KY. Clinical significance of NGAL and KIM-1 for acute kidney injury in patients with scrub typhus. PLoS One. 2017;12:e0175890.
13. Lou W, Cheng Q. Urinary N-acetyl-β-d-glucosaminidase (NAG) levels and risk of cardiovascular events in diabetic patients. Int J Gen Med. 2021;14:10495-502. doi: 10.2147/ijgm.s337874.
14. Shao X, Tian L, Xu W, Zhang Z, Wang C, Qi C, et al. Diagnostic value of urinary kidney injury molecule 1 for acute kidney injury: a meta-analysis. PLoS One. 2014;9:e84131. doi: 10.1371/journal.pone.0084131.
15. Li WW, Huang D, Shen PC, Wu Q, Sun C, Wang XX, et al. [Effects of Gubentongluo Formula on Oxidative Stress Reflected by Expressions of PPARα and L-FABP in Mice with IgA Nephropathy]. Sichuan Da Xue Xue Bao Yi Xue Ban. 2017;48:210-5.
16. Chiang TH, Yo CH, Lee GH, Mathew A, Sugaya T, Li WY, et al. Accuracy of liver-type fatty acid-binding protein in predicting acute kidney injury: a meta-analysis. J Appl Lab Med. 2021. doi: 10.1093/jalm/jfab092.
17. Scherberich JE, Gruber R, Nockher WA, Christensen EI, Schmitt H, Herbst V, et al. Serum uromodulin-a marker of kidney function and renal parenchymal integrity. Nephrol Dial Transplant. 2018;33:284-95. doi: 10.1093/ndt/gfw422.
18. Wuttke M, Li Y. A catalog of genetic loci associated with kidney function from analyses of a million individuals. Nat Genet. 2019;51:957-972. doi: 10.1038/s41588-019-0407-x.
19. Quoc Hoang TA, Tam V, Thang HV. Plasma asymmetric dimethylarginine and its association with some of cardiovascular disease risk factors in chronic kidney disease. Med J Malaysia. 2019;74:209-14.
20. Li C, Shang Guo P, Hui Ying L, Jin JZ, Jin J, Yi Q, et al. SP413 L-Carnitine Treatment Attenuates Renal Tubulointerstitial Fibrosis Induced by Unilateral Ureteral Obstruction. Nephrol Dial Transplant. 2019;34:gfz103.
21. El Sebai AA, El Hadidi ES, Abdel Al H, El Sayed EY. Pentraxin-3 in hemodialysis patients: Relationship to comorbidities. Saudi J Kidney Dis Transpl. 2016;27:701-9. doi: 10.4103/1319-2442.185226.
22. Adler C, Heller T, Schregel F, Hagmann H, Hellmich M, Adler J, et al. TIMP-2/IGFBP7 predicts acute kidney injury in out-of-hospital cardiac arrest survivors. Crit Care. 2018;22:126. doi: 10.1186/s13054-018-2042-9.
23. Nalado AM, Olorunfemi G, Dix-Peek T, Dickens C, Khambule L, Snyman T, et al. Hepcidin and GDF-15 are potential biomarkers of iron deficiency anaemia in chronic kidney disease patients in South Africa. BMC Nephrol. 2020;21:415. doi: 10.1186/s12882-020-02046-7.
24. Kapoula GV, Kontou PI, Bagos PG. Diagnostic Performance of Biomarkers Urinary KIM-1 and YKL-40 for Early Diabetic Nephropathy, in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis. Diagnostics (Basel). 2020;10:999. doi: 10.3390/diagnostics10110909.
25. Ghoul BE, Squalli T, Servais A, Elie C, Meas-Yedid V, Trivint C, et al. Urinary procollagen III aminoterminal propeptide (PIIINP): a fibrotest for the nephrologist. Clin J Am Soc Nephrol. 2010;5:205-10. doi: 10.2215/cjn.06610909.