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Review Article

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# Novel Biomarkers of Kidney Injury: Possibility Toward Preventive Strategies

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## ABSTRACT

Acute kidney injury is a common comorbidity especially observed in hospitalized patients with high morbidity and mortality. However, biomarkers assessing the degree or etiology of acute kidney injury and predictive markers to detect the risk of acute kidney injury or chronic kidney disease progression are lacking. Serum or urinary creatinine, cystatin C, urine albumin-to-creatinine or urine protein-to-creatinine ratio, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, and chitinase 3-like 1 have commonly been utilized as potential markers while all have significant limitations restricting their clinical use. In this review, we aim to evaluate the possible role of selected novel biomarkers including myo-inositol oxygenase, microRNA, cell cycle arrest molecules, Dickkopf-related protein 3, and a few pro-inflammatory cytokines that may have clinical significance by providing a tool for early detection of acute kidney injury or chronic kidney disease progression risk and identify high-risk patients for such medical comorbidity.

**Keywords:** Acute kidney injury, biomarkers, microRNAs, cytokines, chronic kidney disease

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## INTRODUCTION

Acute kidney injury (AKI) is a common diagnosis in hospitalized patients which causes significant morbidity and mortality with approximately 4 million hospitalizations in the United States in 2014.[1] Even though multiple potential serum and urine markers have been hypothesized for the early detection of AKI, assessment for progression risk into chronic kidney disease (CKD) and identify the high-risk populations prior to the development of AKI, all have significant drawbacks. For instance, measurement of serum creatinine is highly limited due to a lack of specificity to disease etiology, an unreliable indicator of estimated glomerular filtration rate (eGFR) due to interference with multiple parameters including diuretic therapy, insensitivity to minor changes in kidney function, being a late marker of kidney damage and dependence to certain clinical variables including age, gender, and muscle mass.[2] Other novel markers of kidney diseases among which few have become clinically available have also major limitations. Neutrophil gelatinase-associated lipocalin (NGAL) measurement is limited due to interference by medical comorbidities such as cancer, atherosclerosis, and Alzheimer's disease and being a poor indicator of AKI in patients with baseline eGFR below <60 mL/min/1.73 $m^2$ while kidney injury molecule-1 (KIM-1) measurement is limited by lack of clear cutoff values and standardized laboratory assays (Table 1).[3] In this review, we aimed to evaluate the selected novel biomarkers of AKI with multiple preclinical and clinical studies which may provide clinical advantages of earlier detection and identification of high-risk populations which may result in a preventive strategy algorithm for such common medical comorbidity.

**Table 1.** Comparison of Major Novel Biomarkers of Kidney Injury with Classical Markers Including Serum Creatinine and Cystatin C

| Novel Biomarkers | Advantages over Classical Biomarkers | Disadvantages over Classical Biomarkers |
|---|---|---|
| Myo-Inositol Oxygenase (MIOX) | • Early marker of kidney injury with the potential to be used as predictive agent<br>• Potential marker for diabetic kidney disease<br>• Potential use in the differentiation of minimal change disease and focal segmental glomerulosclerosis | • Less widely available<br>• Lack of standardized assays or outcome measures<br>• High cost<br>• Influenced by serum glucose concentration, oxidative stress, and levels of free fatty acids |
| Tissue inhibitor of metalloproteinase 2 (TIMP-2)—insulin-like growth factor (IGF) binding protein 7 (IGFBP7) | • Early marker of kidney injury with the potential to be used as predictive agent<br>• More sensitive marker of kidney injury | • Less widely available<br>• Lack of standardized assays or outcome measures<br>• High cost |
| Dickkopf-related protein 3 (DKK-3) | • Early marker of kidney injury with potential to be used as predictive agent<br>• Independent from the baseline kidney function<br>• Predictive marker for contrast-induced kidney injury even in patients with normal baseline kidney function | • Less widely available<br>• Lack of standardized assays or outcome measures<br>• High cost<br>• Scarce data regarding the AKI-to-CKD progression or CKD |



**Myo-Inositol Oxygenase**

A kidney-specific enzyme located at proximal tubules which is the only catabolic pathway for myo-inositol, namely myo-inositol oxygenase (MIOX), has gained potential interest in preclinical and clinical studies conducted on metabolic syndrome, obesity, AKI, and CKD.[4] Even though myo-inositol has shown to be protective against metabolic syndrome and its components including insulin resistance, D-glucuronic acid formed as a result of the enzymatic reaction catalyzed by MIOX is converted into xylulose and ribulose along with the formation of multiple reactive oxygen species (ROS).[5] Myo-inositol oxygenase expression is affected by serum glucose concentration, oxidative stress, and levels of free fatty acids since the promoter responsible for MIOX expression has osmotic response elements, oxidant response elements, sterol response elements, and carbohydrate response elements.[6]

Cisplatin-induced alterations in renal tissue including activation of p53 expression, nuclear translocation, and DNA-binding of NF-κB and formation of ROS are all reduced in MIOX-knockout mice compared to wild-type. Additionally, the markers of kidney injury including serum urea, creatinine, and KIM-1 levels and markers of apoptosis such as Bax, cleaved caspase-3, and NADPH oxidase-4 are reduced according to a study conducted on transgenic mice regarding the role of MIOX on cisplatin-induced AKI. Additionally, MIOX-overexpressing mice have demonstrated the worst clinical, laboratory, and pathological outcomes.[7] Myo-inositol oxygenase-associated AKI has been linked to a process referred to as ferroptosis, a caspase and necrosome-independent mechanism of cell death in which iron overload leading to lipid hydroxylation and peroxidation results in cell death, which is partially reversible by an inhibitor called as ferrostatin-1.[8] Similar findings have also been demonstrated for cadmium-induced AKI on mice subjects in which tubular cell deaths have been linked to necroptosis, attenuated by an inhibitor referred to as necrostatin-1.[9] Such findings not only illustrate the role of MIOX as a marker of AKI but also emphasize the possibility of potential therapeutic intervention point for AKI. Few other animal studies have investigated the role of MIOX in various types of AKI with similar outcomes.[10]

The first study demonstrating the potential prognostic or predictive role of MIOX in AKI has been conducted in 2014 on mice subjects and intensive care unit-admitted patients. Patients are categorized as having AKI and oliguria according to the Kidney Disease: Improving Global Outcome KDIGO criteria in which 42 patients are diagnosed with AKI (23 patients with oliguria and 5 patients with kidney replacement therapy requirement at the time of diagnosis) and 17 patients are categorized as control group. The mean time for serum creatinine increase meeting the AKI diagnostic criteria is 54 hours (±3.8 hours). Plasma MIOX levels are elevated in patients with AKI at time 0 (12.4 ± 4.3 ng/mL) and at 54th hour (10.1 ± 5.3 ng/mL), especially in oliguric AKI patients at time 0 (20.2 ± 7.5 ng/mL) and time 54th hour (17.1 ± 11.0 ng/mL), compared to the control group. The elevation of serum MIOX levels precedes the elevation of serum creatinine by a mean of 54.3 ± 3.8 hours, thus, appears as an early marker of AKI.[11] Another study conducted

## MAIN POINTS

- Novel biomarkers of acute kidney injury which may provide clinical advantages of earlier detection and identification of high-risk populations are needed.
- Myo-inositol oxygenase has a clinical potential to be utilized as a predictive marker for the development of acute kidney injury and acute kidney injury-to-chronic kidney disease transition along with its potential use as a diagnostic tool to differentiate various types of renal diseases.
- Cell-cycle arrest molecules, microRNAs, Dickkopf-related protein-3, monocyte chemoattractant protein-1, proenkephalin, and interleukin-18 are promising markers for AKI.

on a total of 77 patients, 39 AKI patients and 38 healthy controls, demonstrates that serum MIOX levels are a valuable tool to assess AKI with a sensitivity of 53.8% (95% CI; 37.2-69.9) and specificity of 81.5 (95% CI; 65.7-92.3).[12] Furthermore, a 4-month follow-up study of a total of 126 patients treated for community-acquired AKI indicates that serum MIOX level at discharge predicts the risk for progression into CKD with every 10% increase in standardized serum MIOX levels is associated with 13.5% increase in CKD progression risk which is higher compared to NGAL, serum creatinine, and urine protein-to-creatinine ratio.[13]

As MIOX expression is under the influence of serum glucose concentration, it has been hypothesized that MIOX has a potential role in the pathophysiology of diabetic kidney disease (DKD). Myo-inositol oxygenase expression is considerably higher in DKD compared to healthy controls according to a study conducted on 90 patients with type 2 diabetes mellitus and DKD with varying degrees of albuminuria, while serum and urine MIOX levels are correlated with serum HbA1c, urine albumin-to-creatinine ratio, and pathologically detected tubulointerstitial lesions in a statistically significant manner.[14] Single-nucleotide polymorphism study conducted on the MIOX gene and its promoter with 430 subjects illustrates that SNP of *rs761745* at the promoter region is linked to type 1 diabetes mellitus.[15] Additionally, multiple studies investigating the role of MIOX on DKD have been conducted on animal subjects with concurring findings.[16] Although Western blot studies only detect MIOX expression in the kidney proximal tubular epithelium, reverse-transcription-polymerase chain reaction (RT-PCR) and Western immunoblot analyses have detected low levels of MIOX expression in the retinal pigmented epithelium, lens epithelium, and sciatic nerve, all of which are the sites of major microvascular diabetic complications.[17] Such findings indicate that MIOX is not only involved in DKD but rather may be a key component of diabetes and its complications. Additionally, MIOX levels are significantly higher in patients with gestational diabetes mellitus.[18] Similarly, as MIOX expression is affected via serum-free fatty acid and sterol concentration we may hypothesize the potential pathophysiological roles of MIOX in AKI and CKD observed in metabolic syndrome and obesity.[5]

Clinical diagnosis of focal segmental glomerulosclerosis (FSGS) may be challenging in certain circumstances, especially the distinction between minimal change disease (MCD) due to false-negative kidney biopsies. According to a study assessing the metabolic urine profiles of subjects with MCD and FSGS, urinary myo-inositol levels are significantly higher in FSGS patients compared to healthy controls (*P*: .008) and MCD patients (*P*: .042). Additionally, urinary myo-inositol levels are negatively correlated with eGFR value.[19]

Myo-inositol oxygenase level measurement has a clinical potential to be utilized as a predictive marker for the development of AKI and AKI-to-CKD transition along with its potential use as a diagnostic tool to differentiate various types of renal diseases, though, there is a clear need for future large-scale studies to reach for a definitive approach (Figure 1).

**Cell Cycle Arrest Molecules**

The paradigm in the search for AKI markers has shifted from the damage or functional markers into pre-injury phase biomarkers among which cell cycle arrest molecules such as urine tissue inhibitor of metalloproteinase 2 (TIMP-2) and insulin-like growth factor (IGF) binding protein 7 (IGFBP7) which has United States Food and Drug Administration (FDA) approval to be used in AKI with caution.[20] Tissue inhibitor of metalloproteinase 2 is a crucial component of ischemia–reperfusion injury, while IGFBP7 is involved in the regulation of IGF bioavailability, both are involved in G1 cell cycle arrest.[21]



Initial data demonstrating the clinical importance of cell cycle arrest molecules is derived from a 2-step study in which a total of 340 proteins have been assessed in critically ill patients with AKI and then the top 2 markers are compared with the previously known biomarkers in a separate cohort, referred as the Sapphire cohort, which is composed of 744 critically ill adults with no baseline AKI. At the initial study, IGFBP7 and TIMP-2 demonstrate AUC of 0.77 and 0.75 respectively for stage 2-3 AKI within 12-36 hours, combined AUC of 0.80. In the second study IGFBP7xTIMP-2 combination has demonstrated clear superiority ($P$ = .002) compared to known biomarkers of renal injury including urine and plasma NGAL, plasma cystatin-C, and KIM-1, interleukin-18 (IL-18), pi-GST, and L-FABP in the urine.[22] A 2017 meta-analysis study conducted on a total of 9 studies with 1886 subjects demonstrates that IGFBP7-TIMP-2 is a potential marker for AKI with a sensitivity of 0.83 (95% CI: 0.75-0.899, specificity of 0.72 (95% CI: 0.56-0.84) and AUC of 0.86 (95% CI: 0.82-0.88).[21] Another meta-analysis study involving 5 studies with a total of 1619 critically ill patients also validate the potential role of IGFBP7-TIMP-2 as a marker for AKI and demonstrates that cutoff values around 0.3 (ng/mL)/1000 (high sensitivity) and 2.0 (ng/m:)/1000 (high specificity) may be used in clinical practice depending on the purpose such as a method for detection of AKI or ruling out AKI.[23]

Another important beneficiary effect of early detection of AKI development risk is the obtainment of possible therapeutic or preventive windows. In a prospective randomized controlled clinical trial conducted on 100 subjects with admission urinary [TIMP-2] × [IGFBP7] >0.3 (ng/mL) in which patients either received nephrology consultation and intervention or no intervention, it is showed that the early intervention group has a tendency toward lower serum creatinine values ($P$ < .05), higher urine output ($P$ = .08), and lower need for kidney replacement therapy ($P$ = .08) during follow-up of 3 days.[24] In another single-center clinical trial in which patients undergoing cardiovascular surgery are considered as high risk for AKI if they have high

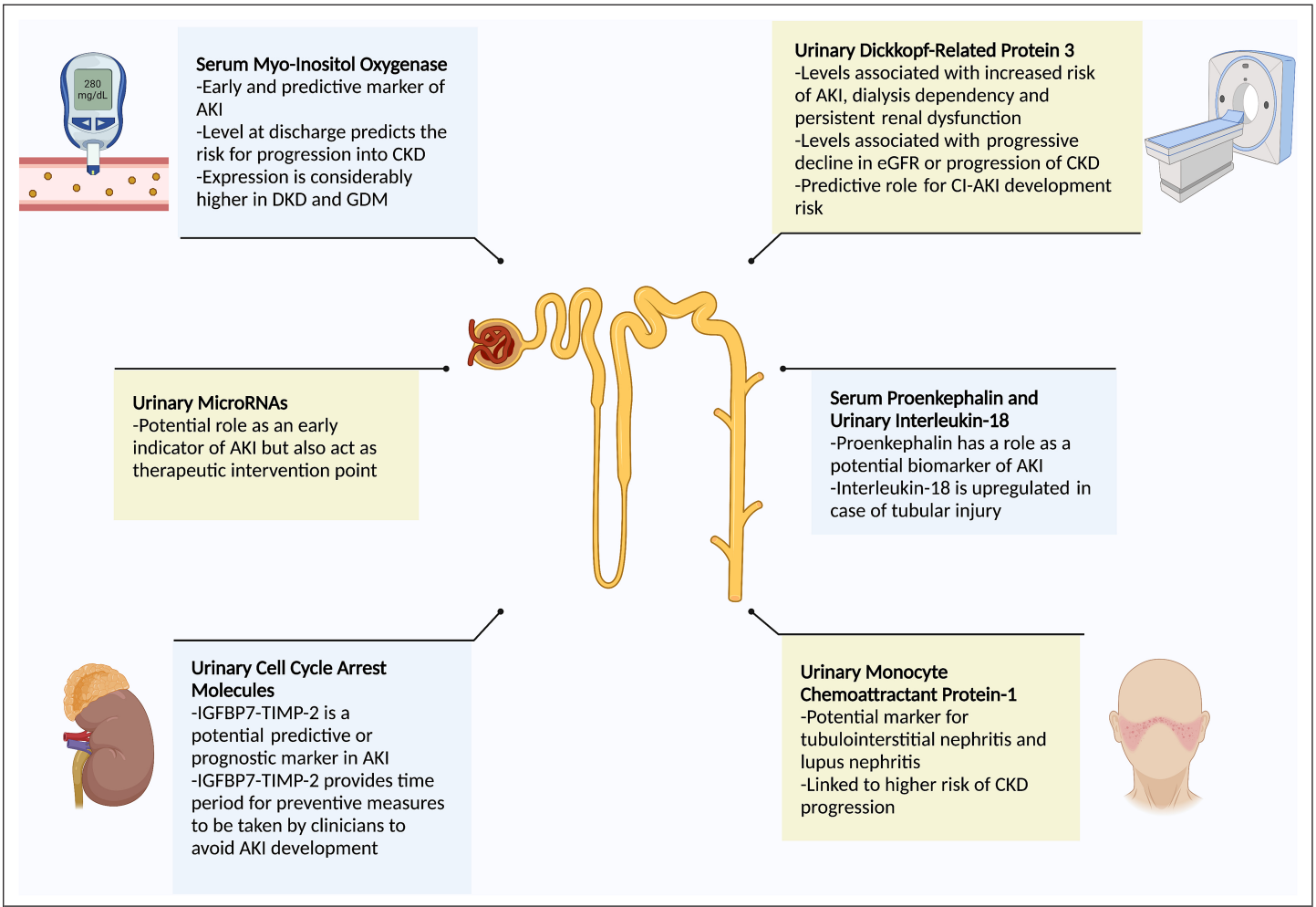


**Figure 1.** General characteristics and potential use of novel biomarkers in clinical practice simplified in a scheme. AKI, acute kidney injury; CI-AKI, contrast-induced acute kidney injury; CKD, chronic kidney disease; DKD, diabetic kidney disease; eGFR, estimated glomerular filtration rate; GDM, gestational diabetes mellitus; IGFBP7, insulin-like growth factor binding protein 7; RNA, ribonucleic acid; TIMP-2, tissue inhibitor of metalloproteinase 2.



levels of urinary [TIMP-2] × [IGFBP7]. The development of AKI ($P$ = .004), hyperglycemia ($P$ < .001), and hemodynamic abnormalities ($P$ < .05) is less commonly encountered in the intervention group in a statistically significant manner, while no differences in terms of the need for kidney replacement therapy or major adverse kidney events at day 30-60-90 have been detected.[25] Another multicenter randomized controlled trial conducted on 278 patients among whom high-risk patients are identified through urinary [TIMP-2] × [IGFBP7] after cardiovascular surgery demonstrated that implementation of supportive measures outlined by the KDIGO guideline reduces the rate of moderate-to-severe AKI in a statistically significant manner ($P$= .034).[26]

We believe that cell cycle arrest molecules, especially IGFBP7-TIMP-2, may become more commonly available and utilized in the clinical practice since it not only acts as a potential predictive or prognostic marker in AKI, but it provides a time period for preventive measures to be taken by clinicians to avoid AKI development.

## MicroRNAs

MicroRNAs are short single-stranded non-coding mRNA types mostly involved in the regulation of protein-coding genes. They have been implicated as part of pathophysiological process in inflammation, apoptosis, fibrosis, necrosis, and certain diseases. Studies investigating the predictive role of microRNAs in AKI are premature and far from reaching a definitive outcome or clinical use. In ischemia–reperfusion injury model study conducted on mice, microRNA-494 levels rise considerably before the rise in serum creatinine levels, and urinary levels of microRNA-494 is approximately 60-fold of the controls. In addition to possibly being an early indicator of AKI, microRNA-494 appears to have certain pathophysiological roles in the development of AKI, including downregulation of activating transcription factor 3 (ATF3) by binding to its 3'untranslated region which results in the activation of NF-κB pathway and its downstream pro-inflammatory pathways.[27] Another study conducted on mice subjects demonstrated 100-fold higher urinary levels of microRNA-16 levels in AKI subjects with ischemia–reperfusion injury which is shown to be caused by binding of

C/enhancer-binding protein-β (C-EBP-β) to promoter region of microRNA-16 which is responsible from the inhibition of anti-apoptotic BCL-2 activity by binding to its 3'untranslated region.[28] On the other hand, microRNA-107 is associated with tumor necrosis factor-alpha (TNF-α) secretion, which may be responsible for the tubulointerstitial damage in septic patients which is reversible by the in vitro inhibition of microRNA-107 on cell lines.[29]

Despite considerable progress has been made in the studies investigating the role of microRNAs in AKI exclusively in cell cultures and animal subjects, the need for future large-scale human studies is clear. However, microRNAs may have the potential to not only be an early indicator of AKI but also act as a therapeutic intervention point due to their role in the disease course.

**Dickkopf-Related Protein 3**

As a glycoprotein secreted from tubular epithelia, mesenchymal cells, and their progenitors, Dickkopf-related protein 3 (DKK-3) is involved in multiple cellular processes including cellular proliferation, differentiation, and apoptosis via the Wnt/β-catenin pathway.[30] Additionally, DKK-3 has been involved in the kidney fibrosis process through the upregulation of Wnt signaling pathway.[31] An observational cohort study conducted on 733 patients undergoing elective cardiac surgeries demonstrates that urinary DKK-3/creatinine ratio over 471 pg/mg is associated with increased risk of AKI ($P$ = .015) regardless of the baseline kidney function, increased risk for dialysis dependency ($P$ = .02), and persistent kidney dysfunction ($P$ = .0072) after a 90-day follow-up period.[32] In another prospective multicenter clinical trial conducted on 2314 patients with a chronic obstructive pulmonary disease with a median follow-up period of 37 months, baseline urinary DKK-3 level has been linked to eGFR decline in the follow-up, while such association is especially apparent in patients with baseline eGFR over 90 mL/min/1.73m$^2$ and without proteinuria.[33] Nonetheless, these parameters may not be in clinical use forthe upcoming years (Figure 1).

Contrast-induced AKI (CI-AKI) is a common clinical concern in diagnostic and therapeutic procedures especially in patients with baseline kidney dysfunction without a reliable indicator of risk analysis. A prospective cohort study conducted on 490 subjects planned to undergo coronary angiography with a 72-hour follow-up period for CI-AKI points out the potential predictive role of urinary DKK-3/creatinine ratio for CI-AKI development risk. Patients who develop CI-AKI within 72 hours of the procedure have 3.8-fold higher pre-procedural urinary DKK3/creatinine ratio ($P$ = .047) even for the patients without baseline CKD. On the other hand, patients who develop CI-AKI demonstrate 29-fold increase in DKK-3/creatinine ratio, considerably lower than the 43-fold increase in patients without CI-AKI.[34] Another study conducted on 458 patients prepared to undergo contrast-requiring cardiovascular procedures demonstrate a similar association between baseline urinary DKK3/creatinine ratio and CI-AKI while also illustrating that elevated urinary DKK3/creatinine ratio is also linked to persistence of such kidney dysfunction over 1-month follow-up period.[35]

**Other Novel Biomarkers**

Multiple other biomarkers for kidney injury and certain kidney diseases have been hypothesized in various clinical trials, however, clinical and pre-clinical evidence for such biomarkers is currently weak.

**Monocyte Chemoattractant Protein-1:** Monocyte chemoattractant protein-1 (MCP-1), a protein involved in the recruitment of most pro-inflammatory cells, has been indicated as a marker for tubulointerstitial nephritis in few small sampled cohort studies and its levels in the urine has been found to be correlated with the degree of lesions on kidney biopsy.[36] Additionally, the urinary level of MCP-1 is proposed as a marker of lupus nephritis according to a clinical study conducted with 109 biopsy-proven lupus nephritis cases compared with healthy controls.[37] Moreover, a study conducted on 37 patients demonstrates that MCP-1 levels are correlated with the macrophages and inflammatory cells in the tubulointerstitial area ($P$ < .005), especially in the cortex but not with the inflammatory cells at glomeruli ($P$ = .19) or cortical tubulointerstitial fibrosis ($P$ = .62).[38] Therefore, MCP-1 appears to be a biomarker of acute inflammatory response at the tubulointerstitial area from which it may be clinically utilized as a tool to differentiate various conditions. Nevertheless, higher levels of MCP-1 have also been linked to a higher risk of CKD progression according to a multicenter prospective cohort study conducted on 1538 hospitalized patients with a median of 4.3 years of follow-up.[39]



**Proenkephalin:** Kidney has the second highest density delta opioid receptors following the central nervous system while the exact physiological role is unknown.[40] Although most enkephalins are bound to plasma proteins and have short half-lives, they are unfit to be used as a marker except proenkephalin which is unbound to plasma proteins and has a relatively long half-life.[41,42] Multiple studies have investigated the role of proenkephalin as a biomarker of AKI in especially critically ill patients with promising outcomes.[43,44] However, there is a clear need for future studies for a better understanding of the pathophysiological and clinical association of proenkephalin and AKI.

**Interleukin-18**

Interleukin-18 is secreted as an inactive peptide that is activated through the actions of caspase-1 and exerts its pro-inflammatory effects through the IL-18 receptor/IL-18 receptor accessory protein heterodimer.[45] Even though IL-18 is produced at the intercalated cells of the collecting ducts of healthy kidneys, its production is upregulated in case of tubular injury.[46] Both IL-18 or caspase-1 knockout mice subjects are resistant to the development of AKI.[47] Nevertheless, studies conducted on human subjects in different clinical settings including emergency departments and intensive care units are unable to show

a reliable predictive role of IL-28 while estimating the risk for AKI development.[48]

### Cytokines and Other Interleukins

Few other cytokines have also been proposed as potential biomarkers of acute tubulointerstitial nephritis, TNF-alpha, and IL-9, following the demonstration of statistically higher levels of both urinary markers in patients with acute interstitial nephritis (AIN) compared to other glomerular or tubular diseases in a cohort of 218 patients with biopsy-proven diagnosis.[49]

## FUTURE PERSPECTIVE

The search for more sensitive and specific early biomarkers of AKI and CKD has been ongoing with multiple clinical trials. Multiple clinical trials that investigate the role of novel biomarkers of kidney injury in various clinical settings including ICU-admitted patients, surgery patients, and patients with sepsis have been conducted (NCT04647396, NCT03856723, NCT05285709, NCT02791880, NCT05310812, NCT04693962, NCT05458063). Few clinical trials investigating the role of biomarkers of CI-AKI (NCT02261909, NCT03004950) and AIN (NCT03836144) are being conducted. Nevertheless, future large-scale randomized clinical trials investigating the role of novel biomarkers as predictive tools in AKI and CKD are required for their wide-scale clinical use.



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