Acute Kidney Injury: Tubular Markers and Risk for Chronic Kidney Disease and End-Stage Kidney Failure

Hon Liang Tan\textsuperscript{b} John Q. Yap\textsuperscript{c} Qi Qian\textsuperscript{a}

\textsuperscript{a}Division of Nephrology and Hypertension Department of Medicine, Departments of \textsuperscript{b}Anesthesiology and \textsuperscript{c}Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN, USA

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Abstract
Acute kidney injury (AKI) is a common clinical syndrome directly related to patient short-term and long-term morbidity and mortality. Over the last decade, the occurrence rate of AKI has been increasing, and there has also been a growing epidemic of chronic kidney diseases (CKD) and end-stage kidney disease (ESRD) linked to severe and repeated episodes of AKIs. The detection and management of AKI are currently far from satisfactory. A large proportion of AKI patients, especially those with preexisting CKD, are at an increased risk of non-resolving AKI and progressing to CKD and ESRD. Proposed pathological processes that contribute to the transition of AKI to CKD and ESRD include severity and frequency of kidney injury, alterations of tubular cell phenotype with cells predominantly in the G2/M phase, interstitial fibrosis and microvascular rarification related to loss of endothelial–pericyte interactions and pericyte dedifferentiation. Innate immune responses, especially dendritic cell responses related to inadequate adenosine receptor (2a)-mediated signals, autophagic insufficiency and renin-angiotensin system activation have also been implicated in the progression of AKI and transitions from AKI to CKD and ESRD. Although promising advances have been made in understanding the pathophysiology of AKI and AKI consequences, much more work needs to be done in developing biomarkers for detecting early kidney injury, prognosticating kidney disease progression and developing strategies to effectively treat AKI and to minimize AKI progression to CKD and ESRD.

Introduction

Acute kidney injury (AKI) is associated with a rise in hospital morbidity and mortality [1, 2], and it continues to impose significant healthcare and economic burdens [3]. Even small acute increases in serum creatinine (sCr) have been shown to have a lasting impact on long-term mortality [4]. A sizeable portion of hospital survivors with AKI do not recover and progress to chronic kidney disease (CKD) and end-stage renal disease (ESRD), requiring maintenance dialysis [5–7]. The incidence rate of renal progression following AKI has been estimated to be 4.9 events/100 patient-years [8] and is particularly increased in the elderly [7]. In a long-term follow-up study of nearly 30,000 cardiac surgery patients, progression to CKD and ESRD was correlated with AKI severity, which peaked in the first 2 years and persisted for 5 years [7].
Despite extensive research, therapeutic interventions to reduce the impact of AKI have not met with much success.

One of the difficulties with effective management of AKI lies in the inability to detect early nephron damage and thus, the inability to precisely identify patients at high risk of progression to CKD/ESRD. Several methods to detect renal injury, such as urine analysis and microscopy [9, 10] as well as fractional urinary excretion of sodium and urea [11, 12] initially showed promise but were subsequently found to be inadequate [13–16]. sCr is the only available and routinely used diagnostic marker for AKI. It is, however, affected by many factors such as age, gender, muscle mass, protein intake, drug metabolism and existing renal reserve and is a late kidney injury marker. Yet, there is continued reliance on sCr measurements in all the definitions of AKI developed by major international committees [17–19].

Better diagnostic tests for detecting early kidney tubular injury would help with timely AKI identification and, thus, allow interventions to halt AKI progression, to deter the development of CKD/ESRD and ultimately to improve outcomes. In this review, we summarize known tubular markers of AKI progression and several postulated mechanisms of CKD/ESRD stemming from AKI.

**Tubular Markers of AKI Progression**

Renal biomarkers can be conceptualized into 2 distinct but related components: first, the detection and localization of injury to the specific section of the kidney; and, second, the quantitation of the resultant kidney dysfunction due to the injury. The former involves the early detection of minute amounts of substances released by injury or upregulated molecules unique to the different cells or cellular pathways within the kidney tubules. The latter involves finding a marker to assess the severity of kidney functional (clearance) decline, in essence, a search for a better marker than sCr. Here, we focus on the current knowledge of tubular markers of renal injury.

**Serum and Urine Kidney Injury Molecule 1**

Kidney injury molecule 1 (KIM-1) is a 38.7-kD transmembrane protein with a very low expression in the normal kidney. Its expression is markedly upregulated after ischemia-reperfusion injury. It reflects the proliferating dedifferentiated epithelial cells of the proximal tubules and appears to peak at approximately 48 h [20]. The extracellular component of KIM-1 is shed from the membrane in a matrix metalloproteinase-dependent manner. In primary cultured kidney cells, KIM-1 expression has been shown to confer a phagocytic phenotype, promoting the phagocytosis of apoptotic cells and necrotic debris, implying a role for KIM-1 in renal recovery and tubular regeneration following AKI. It promotes epithelial regeneration and regulates tubule cell apoptosis. In murine AKI models, KIM-1 over-expression reduces kidney fibrosis and development of ESRD [21]. Persistent KIM-1 elevation in blood, however, indicates ongoing tubular injury, which would be a risk for the development of CKD/ESRD. Urinary KIM-1 shows similar correlation of kidney injury, and one may postulate the use of persistent KIM-1 levels to prognosticate development ESRD. On the other hand, a decline in KIM-1 levels might not necessarily mean that the fibrotic process is not already underway.

**Neutrophil Gelatinase-Associated Lipocalin**

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2 or oncogene 24p3, is a 25-kD protein widely expressed in a variety of adult human tissues, including prostate, salivary gland, stomach, colon, trachea, lung, liver and kidney [22]. Intrarenal NGAL is dramatically upregulated following ischemic or nephrotoxic kidney injury. As early as 3 h following the injury, elevated NGAL protein is detectable in the urine. The major renal source of urinary NGAL is from the thick ascending limb and collecting ducts. Non-specific plasma NGAL can be filtered through glomeruli and be absorbed in the proximal tubules in a megalin-dependent fashion [23]. Thus, urine NGAL elevation could reflect the decreased absorption of filtered NGAL due to dysfunction or injury in the proximal tubules and/or increased NGAL release from the thick ascending limb and collecting ducts [24].

NGAL can bind to iron-siderophore complexes. The NGAL binding to the complex can exert bacterial static function with the sequestering iron-siderophore complex and prevent the complex uptake by bacterial pathogens [25, 26]. Studies in rodent models of AKI have shown that iron-siderophore-loaded NGAL (holo-NGAL) protects the kidney from ischemic reperfusion injury and attenuates the severity of AKI if administered 1 h after reperfusion [27]. Holo-NGAL also upregulates heme oxygenase-1, which is a renal protective enzyme [28]. Thus, holo-NGAL treatment could potentially protect the kidney from worsening AKI.

Urinary NGAL has been examined as a potential marker of kidney disease progression to ESRD in animal...
models [29]. Human studies, though small and few, have demonstrated the possible utility of high baseline levels of plasma and urinary NGAL in predicting progression [30, 31]. The area under the receiver operating characteristic curve (AUC) is estimated at 0.78 for baseline urine NGAL and 0.70 for baseline serum NGAL [22], suggesting potential utility in prognostication post-AKI kidney disease progression.

**Liver-Type Fatty Acid-Binding Protein**

Liver-type fatty acid-binding protein (L-FABP) is a 14-kDa protein expressed in the proximal tubules. Urinary L-FABP levels increase almost immediately and peak within 6 h after tubular injury and correlate strongly with renal ischemic time [32]. One study found significantly higher L-FABP levels in a group of patients who progressed to more severe AKI compared to patients who did not [33]. L-FABP levels are also elevated in patients with known renal disease risk factors of hypertension and diabetes in the absence of overt kidney damage, further enhancing its potential to be used to identify patients at increased risk.

The exact function of L-FABP is yet to be fully elucidated. It is, however, considered a renal protective protein in general. It binds to and promotes the metabolism of fatty acids and possesses antioxidant properties. L-FABP gene contains a hypoxia inducible factor-1α response element. In an L-FABP transgenic mouse model, L-FABP expression at baseline (prior to kidney injury) reduces the severity of renal ischemia-reperfusion injury [34]. Moreover, baseline urinary L-FABP level can be considered an index marker for renal tissue hypoxia and susceptibility to additional insult. L-FABP gene expression is upregulated by peroxisome proliferator-activated receptor-α [35]. Data using proliferator-activated receptor-α agonists to attenuate toxicity and ischemia-associated AKI have, however, generated conflicting results.

**Interleukin-18**

Interleukin-18 (IL-18) is a 22-kD pro-inflammatory cytokine formed in the proximal tubular cells. Urinary IL-18 is elevated following renal injury [36]. IL-18 must be cleaved by caspase-1 as part of a multiprotein complex termed inflammasome. Pyrin domain of the inflammasome contains NOD-like receptor family of proteins. The NOD-like receptor ligands can be grouped into (1) pathogen-associated molecular pattern and (2) damage-associated molecular pattern released from damaged and dying cells [37, 38]. Binding of these molecules to NOD-like receptors results in proteolytic enzyme activation including caspase-1 activation, resulting in IL-18 maturation. The mature IL-18 mediates inflammatory response through upregulating NF-kappa B pathway including TNF-α, iNOS, chemokines MCP-1, and MIP-2, which causes inflammation by attracting microphage and neutrophils [39]. IL-18 worsens tubular necrosis in ischemic-reperfusion [39] and nephrotoxin [40] animal models via Fas/Fas ligand pathways [41]. Disrupting the IL-18 signaling has consistently shown to attenuate kidney injury. Although further studies are mandatory, AKI patients with high urine IL-18 concentration could potentially benefit from anti-IL-18 therapy, although its utility is yet to be validated to prognosticate long-term AKI outcomes.

**Urinary Insulin-Like Growth Factor-Binding Protein 7 and Tissue Inhibitor of Metalloproteinase-2**

Recent Sapphire study, a large validation study of 744 critically ill patients, uncovered 2 novel AKI markers, tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor-binding protein-7 (IGFBP-7), which predicted severe stage 2 or 3 AKI defined by Kidney Disease Improving Global Outcomes definition within 12 h. TIMP-2 [42] and IGFBP-7 are inducers of G1 cell cycle arrest of renal tubular cells, which occurs during the early period of cell injury caused by ischemic or inflammatory processes [43, 44]. Failure to achieve G1 cell cycle arrest can lead to an increased proportion of renal tubular cells in the G2/M phase, which in the animal model shows to be correlated with lasting kidney damage, including extensive glomerulosclerosis and interstitial fibrosis [45]. The accuracy of the 2 markers (TIMP-2 and IGFBP-7) was shown to be significantly greater than the currently known AKI markers [46–50]. Although urinary (TIMP-2) × (IGFBP-7) can aid in AKI risk assessment, one should also recognize that it does not replace clinical judgment for patients across the decrease spectrum. Meersch et al. [51] examined sensitivity and specificity of (TIMP-2) × (IGFBP-7) for AKI (≤stage 1) in an at-risk group of patients undergoing cardiac surgery. They found a sensitivity of 0.92 and specificity of 0.81 for AKI risk prediction using a cutoff value of 0.5 (AUC 0.90) of the maximum urinary (TIMP-2) × (IGFBP-7) in the first 24 h post-surgery. Their cutoff value for an optimal AKI risk assessment differed from prior studies. Thus, specific groups of at-risk patients may require a specific set of combination assessment tools to predict AKI risk with a high precision. Moreover, there are as yet no longer-term studies of these biomarkers on CKD progression.
Pathological Processes Promoting AKI Progression to CKD and ESRD

Progression of CKD or ESRD from AKI implies persistent renal pathology secondary to either continued or repeated cellular injury and/or aberrant repair mechanisms. The precise mechanism of progression from AKI to CKD and ESRD is complex and not completely understood. Currently, sCr, proteinuria and microalbuminuria are used as markers for kidney disease progression. They are unfortunately neither specific nor sensitive. Several pathological processes implicated in kidney disease progression following AKI are summarized below.

Nature of the Injury

While recovery from mild AKI may be complete, it is increasingly recognized that severe or repeated renal injury will result in abnormal repair mechanisms [52], resulting in renal fibrosis, vascular rarefaction, glomerulosclerosis associated with clinical CKD and ESRD [53].

Studies have shown that severe or repeated renal injury regardless of the causes (ischemic, toxic or obstructive) can engender maladaptive response in that tubular epithelial cells arrest at the G2/M phase of cell cycle as opposed to the G1/0 phase under normal conditions. The maladaptive response is associated with increased transforming growth factor-β1 and connective tissue growth factor gene transcription following activation of c-jun NH2-terminal kinase (JNK) signaling, leading to glomerulo-interstitial fibrosis and persistent kidney dysfunction [54]. Glomerulosclerosis is thought to be related to a reduced glomerular blood flow in the setting of progressive capillary loss and tubulointerstitial fibrosis and paracrine signaling from injured tubular cells [55]. This collaborates with studies showing a delayed peak of podocalyxin, a membrane surface protein released by podocytes after initial tubular damage [56]. The maladaptive changes can be successfully reverted (in murine models) by reducing the proportion of G2/M cells [45, 57, 58], giving hope for new therapeutic targets.

Pericytes Dedifferentiation in Interstitial Fibrosis and Microvascular Rarefaction

Pericytes are crucial to microvascular stabilization. Studies have demonstrated that a switch in secretion of vascular endothelial growth factor isomers in the setting of AKI causes pericytes to detach from capillaries and migrate into the interstitium where they differentiate into myofibroblasts, contributing to renal fibrosis [59] and progressive microvascular rarefaction. These changes perpetuate not only local ischemia but also chronic inflammation and progressive loss of kidney function [60]. Blockade of vascular endothelial growth factor and platelet-derived growth factor, another angiogenic molecule associated with fibrosis [61, 62], appears to inhibit this process [63]. In addition to loss of endothelial–pericyte interactions, cytokines and growth factors elaborated from infiltrating monocytes in the setting of AKI also contribute to interstitial fibrosis and capillary rarefaction [64, 65].

Immune Response

Many prior studies have demonstrated the role of proinflammatory mediators [66] and immune cells in AKI, particularly in ischemic [67] and septic [68, 69] models. Recent work suggests that a balance exists with adenosine 2a receptors rich, anti-inflammatory T regulatory cells appearing to attenuate AKI [70, 71]. Murine experiments are also beginning to shed light on the role of the spleen in AKI. In murine models, ultrasound therapy to the spleen [72] or electrical stimulations to the vagus nerve enhance the stimulations to the spleen to a release acetylcholine, which can ameliorate ischemia-reperfusion-induced AKI. Administration of cholinergic agonists, such as nicotine and GTS-21 has also been shown to attenuate AKI, accompanied by decreased proinflammatory cytokine release (e.g. TNF-α, chemokine (C-C motif) ligand 2, and C-X-C motif chemokine 10) by renal cells. A recent study by Bajwa et al. [73] has also shown that bone marrow-derived dendritic cells deficient in sphingosine 1-phosphate receptor 3 can prevent ischemia-reperfusion-induced AKI via a spleen-dependent mechanism and splenic T-regulatory cell expansion. These experimental results are in line with a recent clinical study showing inflammatory cytokine (IL-8), which augments the prediction of kidney recovery and mortality in AKI patients on renal replacement therapy [74].

Autophagy

Autophagy involves the degradation of cytoplasmic components by the lysosome and is believed to be a cellular defense mechanism to conserve energy and improve chances of cell survival, particularly at the PCT. The process appears to be regulated by transforming growth factor-β1 [75] and emerging evidence suggests it exerts a renoprotective role [76], which is possible, at least in part, related to its capability to degrade insoluble collagen I [77]. In support of this notion, a urine peptidome study in CKD patients has demonstrated an inverse correlation between the amount of collagen I and III breakdown products and CKD progression [78, 79].
Asymmetric Dimethylarginine Elevation

Asymmetric dimethylarginine (ADMA) is an amino acid derived from the catabolism of proteins containing methylated arginine residues [80]. Protein arginine methyltransferase type I forms ADMA [81] and dimethylarginine dimethylaminohydrolase (DDAH)-1 metabolizes ADMA [82]. ADMA is a potent endogenous inhibitor of nitric oxide synthase. Under normal conditions, the production of ADMA is balanced by its metabolism by DDAH. Nakayama et al. show that ischemia-reperfusion-elicited oxidative stress contributes to the progression of AKI by promoting tubular necrosis through the elevation of ADMA in the kidney, via oxidative stress-induced proteosomal degradation of DDAH-1 [83]. As ADMA can cause glomerular injury and progressive renal dysfunction [84], it may be viewed both as a biomarker (not strictly a tubular marker) and a direct renal toxin. Strong association has been shown to exist between elevated plasma ADMA levels and progressive kidney injury in a range of pathologies [85–88]. Strategies to reduce ADMA, that is, to enhance DDAH-1 activity or protein expression, may be a potential strategy to retard renal disease progression.

Renin-Angiotensin System Activation

Renin-angiotensin system (RAS) activation, especially intrarenal activation, is shown to drive progression of AKI and transition from acute to chronic kidney injury. Urinary angiotensinogen is considered a novel prognostic marker for AKI. AKI patients with elevated urinary angiotensinogen have been shown to progress to higher stages of AKI and higher mortality rates [89, 90]. In patients with post cardiac surgery AKI, elevated urinary angiotensinogen had an AUC of 0.75 for predicting progression of AKI to stage 3 and predicting mortality [91]. An animal study shows that spironolactone treatment in animals with AKI reduces progression to CKD [92]. Elevated urinary angiotensinogen marks intrarenal RAS activation and CKD progression [93–95]. Animal studies show that intrarenal angiotensin II increases after renal ischemia-reperfusion injury, while concentrations of angiotensin 1–7 (inhibitory molecule to angiotensin II) decrease in the kidney tissues [96]. Measurement of urinary angiotensinogen could therefore help with identifying AKI patients who are at risk of developing accelerated CKD and could potentially benefit from RAS blockade agents.

Summary and Future Directions

AKI can be caused by a myriad of renal and systemic insults. Mounting evidence suggests the utility of biomarkers in understanding the AKI pathophysiology and predicting AKI outcomes. Understanding the interconnecting processes between AKI and CKD/ESRD will help with identifying at-risk patients for kidney disease progression. A number of promising kidney injury markers are just beginning to be understood, and modulators of their expression are being defined. Further, structure-function studies and studies of genetically-engineered animals that over- or under-express tubular markers and pathological disease-progression processes will provide new insights into their actions and may lead to the development of novel therapies for preventing AKI from progressing to CKD and ESRD.

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References


