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Biomarkers of Acute Kidney Injury

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Abstract

Acute kidney injury (AKI) is a common condition with a high risk of death. The standard metrics used to define and monitor the progression of AKI, such as serum creatinine and blood urea nitrogen levels, are insensitive, nonspecific, and change significantly only after significant kidney injury and then with a substantial time delay. This delay in diagnosis not only prevents timely patient management decisions, including administration of putative therapeutic agents, but also significantly affects the preclinical evaluation of toxicity thereby allowing potentially nephrotoxic drug candidates to pass the preclinical safety criteria only to be found to be clinically nephrotoxic with great human costs. Studies to establish effective therapies for AKI will be greatly facilitated by two factors: (a) development of sensitive, specific, and reliable biomarkers for early diagnosis/prognosis of AKI in preclinical and clinical studies, and (b) development and validation of high-throughput innovative technologies that allow rapid multiplexed detection of multiple markers at the bedside.

Keywords

acute renal failure; clusterin; cystatin-C; cysteine-rich protein-61 (CYR-61); ELISA; Interleukin-18 (IL-18); kidney injury molecule-1 (Kim-1); microfluidics; nanotechnology; neutrophil gelatinase-associated lipocalin (NGAL)

ACUTE KIDNEY INJURY

Definition and Prevalence

Acute kidney injury (AKI) is currently recognized as the preferred nomenclature for the clinical disorder formally called acute renal failure (ARF). This transition in terminology served to emphasize that the spectrum of disease is much broader than that subset of patients who experience failure requiring dialysis support (1). This new nomenclature underscores the fact that kidney injury exists along a continuum: The more severe the injury, the more likely the overall outcome will be unfavorable. The Acute Kidney Injury Network (AKIN), which was formed recently in an effort to facilitate improved care of patients who are at risk for AKI, described AKI as “functional or structural abnormalities or markers of kidney damage

including abnormalities in blood, urine, or tissue tests or imaging studies present for less than three months.” AKI is associated with the retention of creatinine, urea, and other metabolic waste products that are normally excreted by the kidney. Although severe AKI may result in oliguria or even anuria, urine volume may be normal or even increased (2).

Recent epidemiologic data suggest that the progress observed in the understanding of the pathophysiology of AKI and in the clinical care of patients with AKI has failed to yield commensurate improvements in clinical outcomes. AKI has been reported to complicate 1% to 7% (3,4) of all hospital admissions and 1% to 25% of intensive care unit (ICU) admissions (5). Over the past 50 years, mortality rates of patients with AKI in the ICU have remained high, at approximately 50% to 70%. Although there is some indication that mortality rates may be falling, the incidence of AKI has increased greatly over time (6). A recent large international study of the epidemiology and outcome of AKI in critically ill adult patients reported an overall in-hospital mortality rate of 60% (7). Of those who survived to hospital discharge, 13% remained dialysis-dependent. In a smaller retrospective study of 267 adult AKI survivors requiring acute renal replacement therapy, renal insufficiency persisted in 41% and overall 5-year survival postdischarge was 50% (8).

Pathophysiology and Mechanisms

Acute kidney injury can result from decreased renal or intrarenal perfusion, a toxic or obstructive insult to the renal tubule, tubulointerstitial inflammation and edema, or primary reduction in the filtering capacity of the glomerulus (9). Ischemia and toxins, often in the setting of sepsis, account for the largest number of cases of AKI. Ischemia and toxins often combine to cause AKI in severely ill patients with conditions such as sepsis, hematologic cancers, or the acquired immunodeficiency syndrome. It is estimated that 19%–33% of in-hospital AKI cases are attributed to drug nephrotoxicity (10,11). Antibiotics, in particular aminoglycosides such as gentamicin and tobramycin, were the most frequently cited nephrotoxic drugs, followed by analgesics, NSAIDs, and contrast media. Other compounds that can potentially result in AKI include chemotherapeutic agents such as cisplatin; immunosuppressants such as cyclosporine and tacrolimus; environmental contaminants such as cadmium, mercuric chloride, and aristolochic acid; heme pigments; and myeloma light-chain proteins.

A number of pathophysiological mechanisms can contribute to AKI following an ischemic or toxic insult. These include (a) alterations in renal perfusion resulting from loss of autoregulation and increased renal vasoconstriction, (b) tubular dysfunction and cell death by apoptosis and necrosis, (c) desquamation of viable and dead cells contributing to intratubular obstruction, (d) metabolic alterations resulting in transport abnormalities that can lead to abnormalities of tubuloglomerular balance, and (e) local production of inflammatory mediators resulting in interstitial inflammation and vascular congestion (12,13). The processes of injury and repair to the kidney epithelium are depicted schematically in Figure 1. On a cellular level, injury results in rapid loss of cytoskeletal integrity and cell polarity, with mislocalization of adhesion molecules and other membrane proteins such as the Na^+K^+ ATPase and β -integrins, shedding of the proximal tubule brush border, as well as apoptosis and necrosis (14). With severe injury, viable and nonviable cells are desquamated, leaving regions where the basement membrane remains as the only barrier between the filtrate and the peritubular interstitium. This allows for backleak of the filtrate, especially under circumstances where the pressure in the tubule is increased owing to intratubular obstruction resulting from cellular debris in the lumen interacting with proteins such as fibronectin that enter the lumen (15). This injury to the epithelium results in the generation of inflammatory and vasoactive mediators, which can act on the vasculature to worsen the vasoconstriction and inflammation. Thus, inflammation contributes in a critical way to the pathophysiology of AKI (16). In contrast to the heart or brain, the kidney efficiently restores cells that were lost owing to an ischemic or toxic insult

that results in cell death, although it is becoming increasingly recognized that there are longer-term detrimental effects of even brief periods of AKI (17).

Surviving cells that remain adherent contribute to repair. The kidney has the potential to recover a large amount of pre-insult renal function. Whether there is a subpopulation of stem or progenitor cells is a matter of active study at this point in time (18). When the kidney recovers from acute injury it relies on a sequence of events, including epithelial cell spreading and migration to cover the exposed areas of the basement membrane, cell de-differentiation and proliferation to restore cell number, followed by differentiation, which results in restoration of the functional integrity of the nephron (19). The contribution of nontubular progenitor cells to this repair of the tubules is likely to be minimal if any at all (19a). Several studies suggest that there is a very delicate and dynamic relationship between tissue repair and progression or regression of renal injury. A delay or inhibition of nephrogenic tissue repair appears to lead to progression of injury ultimately leading to chronic kidney disease, whereas timely tissue repair may arrest progression of injury, resulting in regression of injury and paving the way for recovery (20).

Diagnosis

Historically, authors have used various measures to assess renal function and define abnormal function to guide diagnosis. It is estimated that more than 30 different definitions of acute renal failure (now termed AKI) exist in the published literature (21), ranging from severe (ARF requiring dialysis) to mild (modest observable increases in serum creatinine) (22). As a result of the disparate clinical and physiologic endpoints used to guide investigation, epidemiologic studies as well as trials of prevention and intervention are often not comparable. As part of the Acute Dialysis Quality Initiative (ADQI) 2nd International Consensus Conference, the RIFLE classification scheme (risk of kidney dysfunction, injury to the kidney, failure of kidney function, loss of kidney function, and end-stage kidney disease) was derived to provide standardized criteria for defining ARF (22a). In recognition of increasing data suggesting that even small changes in serum creatinine are associated with poorer outcome as measured by mortality, AKIN defined AKI as “an abrupt (within 48 hours) reduction in kidney function currently defined as an absolute increase in serum creatinine of either ≥ 0.3 mg/dl (≥ 25 micromole/L) or a percentage increase of $\geq 50\%$ or a reduction in urine output (documented oligouria of <0.5 ml/kg/hr for >6 hours)” (23). The RIFLE criteria were modified so that the risk criteria included an absolute increase in serum creatinine ≥ 0.3 mg dl^{-1} with the new diagnostic criteria in mind (Table 1).

One prevailing weakness with the definition is that it is still entirely based on an increase in serum creatinine or decrease in urine volume; unfortunately, creatinine is a suboptimal marker following injury, when levels are often not reflective of glomerular filtration rate (GFR) owing to a number of renal and nonrenal influences on creatinine levels. In the setting of AKI, the delay between changes in serum creatinine and changes in GFR inhibits the ability to accurately estimate timing of injury and severity of dysfunction following injury (24). A sudden fall in GFR to a constant low level causes a gradual increase in serum creatinine until a new steady state between generation and excretion is achieved. The rate of rise of serum creatinine following AKI is dependent on many factors, including the new GFR, rate of tubular secretion, rate of generation, and volume of distribution (24,25). As a result, large changes in GFR may be associated with relatively small changes in serum creatinine in the first 24–48 h following AKI, resulting not only in delayed diagnosis and intervention but also in underestimation of the degree of injury. In addition, there is considerable variability among patients in the correlation between serum creatinine and baseline GFR, in the magnitude of functional renal reserve, and in creatinine synthesis rates. As a result, a renal injury of comparable magnitude may result in disparate alterations in creatinine concentration in different individuals (26).

There is an urgent need for better biomarkers to permit more timely diagnosis of AKI, prediction of injury severity, and safety assessment during drug development. Better biomarkers will help drug developers make more informed decisions about which products to move forward in testing, which doses to test, and how to design clinical trials that will provide clear information about product benefit and safety. Results from interventional trials suggesting lack of efficacy of putative therapies of AKI are, by definition, confounded by delayed diagnosis and treatment. This paradigm of late recognition and/or treatment attempts is analogous to the futility of initiation of therapy directed toward acute ischemia in patients with myocardial infarction or stroke 48 h after the onset of ischemia (27).

BIOMARKERS OF ACUTE KIDNEY INJURY

Accessible markers of AKI can be components of serum or urine or can be imaging studies or any other quantifiable parameter. The urine has yielded the most promising markers for the early detection of AKI and further characterization is anticipated, which will qualify these markers as useful tools for the earlier diagnosis, identification of mechanism of injury, and assessment of site and severity of injury (Figure 2, Table 2). Hopefully, one or more of these biomarkers, either alone or in combination, will prove to be useful in facilitating early diagnosis, guiding targeted intervention and monitoring disease progression and resolution.

N-acetyl- β -glucosaminidase

N-acetyl- β -glucosaminidase (NAG), a proximal tubule lysosomal enzyme, has been extensively studied and has proven to be a sensitive, persistent, and robust indicator of tubular injury. Increased NAG levels have been reported with nephrotoxicant exposure (28), delayed renal allograft function, chronic glomerular disease, diabetic nephropathy (29), as well as following cardiopulmonary bypass procedures (30). Westhuyzen et al. (31) reported that urinary NAG levels (in addition to other tubular enzymes) were highly sensitive in detecting AKI in a population of critically ill adult patients, preceding increases in serum creatinine by 12 h to 4 days. Chew et al. (32) reported a poorer outcome [death in hospital, requirement for long-term renal replacement therapy (RRT)] in patients with higher urinary NAG levels on admission to a renal care unit. The higher the urinary NAG concentrations in patients already diagnosed using AKI clinical criteria, the greater the incidence of the combined endpoint of dialysis or death (33). The two advantages of using NAG are (a) sensitivity, subtle alterations in the epithelial cells in the brush border of the proximal tubules result in shedding of NAG into the urine and the amount of shed enzyme can be directly correlated to tubular injury; and (b) quantitation, simple and reproducible enzymatic assays are well established to measure the analyte colorimetrically using a spectrophotometer. The disadvantage is that urinary NAG activity has been found to be inhibited by endogenous urea (34) as well as a number of nephrotoxicants and heavy metals (35). In addition, increased urinary NAG levels have been reported in a variety of conditions in the absence of clinically significant AKI, including rheumatoid arthritis (35a), impaired glucose tolerance (35b), and hyperthyroidism (35c). As a result, nonspecificity may limit the use of NAG levels as a biomarker of AKI.

β_2 -microglobulin

β_2 -microglobulin (β_2 M) is an 11.8-kDa protein that is the light chain of the major histocompatibility class (MHC) I molecule expressed on the cell surface of all nucleated cells. β_2 M dissociates from the heavy chain in the setting of cellular turnover and enters the circulation as a monomer (36). β_2 M is typically filtered by the glomerulus and almost entirely reabsorbed and catabolized by the proximal tubular cells (37), a process that may be impeded in AKI. Increased urinary β_2 M excretion has been observed to be an early marker of tubular injury in a number of settings, including nephrotoxicant exposure (38), cardiac surgery (39), and renal transplantation (40), preceding rises in serum creatinine by as many as 4–5 days

(36). Unfortunately, the utility of β_2 M as a biomarker has been limited by its instability in urine, with rapid degradation observed at room temperature and in urine with a pH less than 6.0 (40a). Schaub et al. (40) recently identified cleaved urinary β_2 M as a potential biomarker of tubular injury in renal allografts; however, assays for protein quantification have not been developed. It should also be noted that although β_2 M may serve as an early biomarker for AKI, it has been found to be poorly predictive of severe injury requiring RRT (41).

α_1 -microglobulin

α_1 -microglobulin (α_1 M) is a 27–33-kDa protein synthesized by the liver with approximately half of the circulating protein complexed to IgA. The free form is readily filtered by the glomerulus and reabsorbed by proximal tubule cells. Unlike β_2 M, urinary α_1 M is stable over the range of pH found in routine clinical practice, making it a preferred marker of tubular proteinuria in human bioassays (28). It has been found to be a sensitive biomarker for proximal tubular dysfunction even in the early phase of injury when no histologic damage is observable (42). In a heterogeneous population of patients with nonoliguric AKI, Herget-Rosenthal et al. reported α_1 M to be an early indicator of unfavorable outcome (requirement for RRT) (41). In addition, urinary α_1 M has been proposed to be a useful marker of tubular dysfunction even in low-gestational-age preterm infants, a population at high risk for AKI (43). Although sensitive immunoassays have been developed for the quantification of α_1 M, international standardization is lacking. In addition, a number of conditions have been associated with altered plasma/serum levels, including liver disease, HIV, and mood disorders (43a), and therefore urinary specificity and sensitivity for AKI may be suboptimal in these settings.

Retinol Binding Protein

Retinol binding protein (RBP) is a 21-kDa protein that is hepatically synthesized and responsible for transporting vitamin A from the liver to other tissues. It is freely filtered by the glomerulus and subsequently reabsorbed and catabolized by the proximal tubule. Bernard et al. (44) monitored patients with AKI from various etiologies and found urinary RBP to be a highly sensitive indicator of renal tubule dysfunction, preceding urinary NAG elevation. They reported RBP and β_2 M levels to be highly correlated when urinary pH > 6.0, with progressively increasing RBP/ β_2 M ratios as urinary pH declined, reflecting RBPs stability in acidic urine when compared with the instability of β_2 M (44). In addition, Roberts et al. (45) reported that increased RBP levels during the first two days of life were predictive of clinically significant AKI in infants following birth asphyxia, a setting where interpretation of serum creatinine is particularly problematic as it reflects maternal serum concentration to a significant extent. An increased level of urinary RBP has been reported to be an early diagnostic marker of renal injury in cisplatin-, lead-, mercury-, cadmium-, and cyclosporine-induced nephrotoxicity in patients (45a). Serum RBP levels are depressed in vitamin A deficiency, and urinary levels may theoretically yield a false negative result in this setting (36). RBP, β_2 M, and α_1 M levels in the urine are measured by immunonephelometric methods using a nephelometer. The utility of low-molecular-weight filtered proteins, such as RBP, β_2 M, α_1 M, Cystatin C, and microalbumin (see below), as biomarkers in the setting of AKI is limited by concomitant significant glomerular proteinuria or hyperfiltration, situations where the tubular reabsorptive pathways may be saturated (45b). Furthermore, specificity for AKI is suboptimal.

Cystatin-C

Cystatin-C (Cys-C) is a 13-kDa protein that was initially known as interalpha γ -trace, post- γ -globulin, and gamma-CSF and is believed to be one of the most important extracellular inhibitors of cysteine proteases. Serum concentrations appear to be independent of sex, age, and muscle mass. Cys-C is freely filtered by the glomerulus, reabsorbed and catabolized, but not secreted, by the tubules. Over the past decade, serum Cys-C has been extensively studied

and found to be a sensitive serum marker of GFR and a stronger predictor than serum creatinine of risk of death and cardiovascular events in older patients (46). The only rodent study in which Cys-C was measured was in the rat model of end-stage renal failure in which sequential bilateral nephrectomy was carried out seven days apart. The kinetics of changes in serum Cys-C and creatinine concentrations mimicked the clinical condition (47). Urinary Cys-C levels have been found to be elevated in individuals with known tubular dysfunction (48,49). In addition, Herget-Rosenthal et al. reported that elevated urinary Cys-C levels were highly predictive of poor outcome (requirement for RRT) in a heterogeneous group of patients with initially nonoliguric AKI (50). The measurement of serum Cys-C before 1994 was performed by using an enzyme-amplified single radial immunodiffusion technique that required at least 10–20 h and had a relatively high coefficient of variation (>10%). Subsequently, automated rapid particle-enhanced immunoturbidimetric and immunonephelometric methods were developed that were more precise and were thus approved by the FDA (51). Recently, an automatic quantitative assay to measure urinary Cys-C has also been developed using an N-Latex Cystatin-C kit with a nephelometer (48).

Microalbumin

Microalbuminuria, defined as the pathologic excretion of urinary albumin at levels (30 to 300 mg/L) below the threshold of detection by conventional urinary dipstick, has long been established as a useful marker of the development and progression of renal disease, particularly diabetic nephropathy. Historically, microalbuminuria has been assumed to result from alterations in glomerular filtration secondary to changes in intraglomerular pressure and/or structural changes of the podocyte or glomerular basement membrane. Recent evidence in rats, however, suggests that the normal glomerular filter actually may leak albumin at higher levels than previously thought, and albuminuria may result from failure of the proximal tubule cell retrieval pathway (48a). Microalbuminuria may prove to be a useful marker of AKI and concomitant proximal tubular cell damage. Microalbuminuria has previously been reported with short- and long-term administration of nephrotoxic chemotherapeutics such as cisplatin, ifosfamide, and methotrexate (48b,48c), as well as antibiotics such as gentamicin (48d). Using microalbuminuria as a marker, Leven et al. (48e) demonstrated that N-acetylcysteine may attenuate contrast-induced glomerular and tubular injury. Microalbuminuria, however, may also be caused by vigorous exercise, hematuria, urinary tract infection, and dehydration. Additional studies are necessary to further characterize microalbuminuria in the setting of AKI, especially with respect to its sensitivity and specificity.

Kidney Injury Molecule-1

Kidney injury molecule-1 (KIM-1) is a type I cell membrane glycoprotein containing a unique six-cysteine immunoglobulin-like domain and a mucin domain in its extracellular region. Rat and human cDNAs encoding KIM-1 (Kim-1 in the rodent) were initially identified by our group using representational difference analysis, a polymerase chain reaction–based cDNA subtraction analysis designed to identify genes with differential expression between normal and regenerating kidneys following ischemia/reperfusion (I/R) injury (52). KIM-1 mRNA levels increase more than any other known gene after kidney injury. We have found that the ectodomain of KIM-1 is shed from cells *in vitro* (53) and *in vivo* into the urine in rodents (54) and humans (55) after proximal tubular injury (Table 3). In preclinical and clinical studies using several mechanistically different models of kidney injury, urinary Kim-1 serves as an earlier diagnostic indicator of kidney injury when compared with any of the conventional biomarkers, e.g., plasma creatinine; BUN; glycosuria; increased proteinuria; or increased urinary NAG, γ -GT, or AP levels (54,55). We first developed an enzyme-linked immunosorbent assay (ELISA) to measure Kim-1 in rodent and human urine samples, and recently we have developed a more sensitive, high-throughput microbead-based assay to

quantitate Kim-1 in rat urine (56). This assay has a greater dynamic range and requires less urine volume (30 μ l) and reagents than the conventional ELISA.

In recently completed studies of eight mechanistically different proximal tubule nephrotoxics and two different hepatotoxics in rats, Kim-1 performed very well in identifying proximal tubular toxicity, using histopathology as the gold standard; of 21 urinary markers studied, Kim-1 was found to have the highest sensitivity and specificity (57). Another study demonstrated Kim-1 to be an outstanding biomarker for cadmium nephrotoxicity (57a). Human studies of urinary KIM-1 for the diagnosis of AKI are promising. Han et al. demonstrated marked expression of KIM-1 in kidney biopsy specimens from six patients with acute tubular necrosis, and found elevated urinary levels of KIM-1 within 12 h after an initial ischemic renal insult, prior to the appearance of casts in the urine (55). Liangos et al. studied urinary KIM-1 and NAG in 201 patients with established AKI and demonstrated that elevated levels of urinary KIM-1 and NAG were significantly associated with the clinical composite endpoint of death or dialysis requirement, even after adjustment for disease severity or comorbidity (57b). Van Timmeren et al. stained for KIM-1 protein in tissue specimens from 102 patients who underwent kidney biopsy for a variety of kidney diseases and showed that a positive KIM-1 staining in dedifferentiated proximal tubular cells correlated with tubulointerstitial fibrosis and inflammation and in subset of patients who underwent urine collection near the time of biopsy urinary KIM-1 levels correlated with tissue expression of KIM-1 (57c). The temporal expression pattern and predictive potential of urinary KIM-1 as compared with other markers in various forms of AKI are subjects of ongoing clinical studies.

Clusterin

Clusterin is a multifaceted glycoprotein that was first isolated from ram rete testes fluid by Blaschuk, Burdzy, and Fritz in 1983 and so named because of its ability to elicit clustering of Sertoli cells (58). Clusterin is induced in the kidney and urine of rats, dogs, and primates after various forms of preclinical AKI such as ischemia/reperfusion injury (59,60), toxicant-induced kidney injury, unilateral ureteral obstruction, or subtotal nephrectomy (61-63). Clusterin, like Kim-1, is expressed on the dedifferentiated tubular cells after injury and is also induced in polycystic kidney disease (64) and renal cell carcinoma (65). The nuclear form of human clusterin (nCLU) is proapoptotic and the secretory form (sCLU), which is upregulated in response to any molecular stress, is antiapoptotic and prosurvival. There are recent findings that drugs targeting sCLU expression, using antisense oligonucleotides or short interfering double-stranded RNA, may become promising tools for cancer therapy, especially in treatment of cancers that overexpress sCLU, such as kidney, prostate, colon, breast, and lung tumors (65a). Clusterin is measured by radioimmunoassay, and the amount of protein has been correlated with the elevation of serum creatine and urinary NAG in a gentamicin-induced renal injury model in rats (66). Clusterin mRNA and protein levels, however, did not increase until day 5 in a cisplatin-induced renal injury model (67). To date, there is no clinical study demonstrating the use of clusterin as an early diagnostic/prognostic indicator of acute kidney injury in humans.

Neutrophil Gelatinase-Associated Lipocalin

Human neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein initially identified bound to gelatinase in specific granules of the neutrophil. NGAL is synthesized during a narrow window of granulocyte maturation in the bone marrow (68), but also may be induced in epithelial cells in the setting of inflammation or malignancy (69). The lipocalin superfamily comprises proteins that are composed of 8 β -strands that form a β -barrel enclosing a calyx (69a). The calyx binds and transports low-molecular-weight chemicals. NGAL binds the siderophore (enterochelin) in the calyx with high affinity (0.4 nM), and the siderophore traps iron with a high affinity (10^{-49} M) with a stoichiometry of protein:siderophore:Fe of

1:1:1 (69b). Cowland & Borregaard demonstrated varying degrees of NGAL gene expression in a number of other human tissues, including the uterus, prostate, salivary gland, lung, trachea, stomach, colon, and kidney (70).

NGAL was identified as being one of the seven genes whose expression was up-regulated more than tenfold within the first few hours after ischemic renal injury in a mouse model (71). Although it was shown that exogenous administration of NGAL protected against ischemic kidney injury in mice (72), lipocalin-2 knockout mice do not exhibit increased sensitivity to bilateral renal ischemia/reperfusion injury (73). NGAL is upregulated and can be detected in the kidney (74) and urine of mice three hours after cisplatin (20 mg kg⁻¹) administration and has been proposed as an early biomarker for diagnosing AKI (75). A prospective study of pediatric patients undergoing cardiopulmonary bypass for cardiac corrective surgery found urinary NGAL to be a powerful early marker of AKI, preceding any increase in serum creatinine by 1–3 days. A similar study of adult patients showed urinary NGAL levels at 1, 3, and 18 h after cardiac surgery to be significantly higher in patients who went on to develop clinically significant AKI (76). A retrospective analysis of urine samples from patients with diarrhea-associated hemolytic uremic syndrome revealed that normal urinary NGAL excretion during the early stages of hospitalization had a high negative predictive value of the need for dialysis; however, high urinary NGAL levels were not a reliable predictor of need for dialysis (77). It should be noted that serum NGAL levels are known to rise in the setting of a number of inflammatory and infective conditions and NGAL filtered by the glomerulus appears in the urine (78,79). Further studies are required to determine specificity of urinary NGAL for AKI in the setting of sepsis, a condition frequently associated with clinically significant renal injury.

Interleukin-18

Interleukin-18 (IL-18) is a cytokine that has been identified as an interferon- γ (IFN- γ)-inducing factor in livers of mice treated with propionibacterium acnes and lipopolysaccharide (80). IL-18 activity has been described in a number of inflammatory diseases across a broad range of tissues, including inflammatory arthritis, multiple sclerosis, inflammatory bowel disease, chronic hepatitis, systemic lupus erythematosus, and psoriasis (81). The precursor form of IL-18 (24 kDa) is enzymatically cleaved by IL-1 β -converting enzyme to produce mature 18-kDa IL-18 protein (82). Renal IL-18 mRNA levels have been shown to be significantly upregulated following ischemia-reperfusion injury, inflammatory/autoimmune nephritis, and cisplatin-induced nephrotoxicity (82a).

Urinary IL-18 levels are elevated in patients with AKI and delayed graft function compared with normal subjects and patients with prerenal azotemia, UTI, chronic renal insufficiency, and nephrotic syndrome (83). Median urinary IL-18 levels were 985 pg mg⁻¹ creatinine on day 1 in patients with delayed graft function, compared with 56 pg mg⁻¹ in patients with prompt graft function. Immunohistochemical staining of renal transplant protocol biopsies revealed constitutive IL-18 expression in the distal tubular epithelium. There was strong positive immunoreactivity in the proximal tubules of patients with acute rejection. There was also strong immunoreactivity in infiltrating leukocytes and endothelium, suggesting upregulation in the setting of immunopathological reactions (84). In a study of critically ill adult patients with acute respiratory distress syndrome (ARDS), increased urinary IL-18 was found to be an early marker of AKI, preceding changes in serum creatinine by 1–2 days, and was an independent predictor of death (85). In these studies, IL-18 was measured with an ELISA using commercially available antibodies.

Cysteine-Rich Protein

Cysteine-rich protein (Cyr61) is a cysteine-rich, heparin-binding protein that is secreted and is associated with cell surface and the extracellular matrix, biochemical features that resemble

the Wnt-1 protooncogene and a number of growth factors (86). Cyr61 is a novel ligand for integrins, and signaling through integrin receptors such as $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_6\beta_1$ regulates angiogenesis and tumorigenesis (87). Cyr61 is rapidly induced in the proximal straight tubules of the kidney within 3–6 h after bilateral renal ischemia in the rats. It is also secreted in the urine within 3–6 h and has been proposed as a potential biomarker for AKI in preclinical and clinical studies (88). There are two main limitations for the use of Cyr61 as a biomarker. Urinary Cyr61 levels are reduced over time despite the continuous progression of injury in the bilateral I/R injury model. Cyr61 has been quantitated in the urine by immunoblotting, which is not a high-throughput and sensitive method of measurement (88). Owing to these limitations, there are few preclinical and clinical studies to define the diagnostic capability of this molecule.

Osteopontin

Osteopontin (OPN) is also known as the 44-kDa bone phosphoprotein, sialoprotein I, secreted phosphoprotein I, uropontin, and early T-lymphocyte activation-1 (Eta-1) (89). OPN is synthesized at the highest levels in bone and epithelial tissues. OPN is found at very high levels in human urine ($21.4 \pm 6.2 \text{ mg g}^{-1}$ of creatinine or $1.9 \mu\text{g ml}^{-1}$) and is postulated to act as an inhibitor of calcium oxalate formation, helping to prevent mineral precipitation and stone formation (89a). OPN is also associated with a number of other functions, including regulation of osteoclast function during bone formation, tumorigenesis and transformation, and accumulation of macrophages (89b). OPN is expressed in normal mouse and human kidneys, where it is primarily restricted to the thick ascending limbs of the loop of henle and the distal convoluted tubules (90). OPN mRNA and protein were overexpressed in renal biopsies from patients with essential hypertension with decompensated arteriosclerosis in association with expression of α -smooth muscle actin by interstitial fibroblasts and increased type IV collagen deposition (91). Similar correlations between OPN induction and inflammation and tubulointerstitial fibrosis were observed with human progressive idiopathic membranous nephropathy, crescentic glomerulonephritis, IgA nephritis, and diffuse proliferative lupus nephritis (92–94). OPN is also significantly upregulated in rodent models of kidney injury/disease with injury secondary to I/R injury, gentamicin, cisplatin, cyclosporine, sevoflurane, angiotensin II-induced tubulointerstitial nephritis, puromycin-induced glomerulonephritis, anti Thy-1 nephritis, passive heyman nephritis, protein-overload models of fibrosis, unilateral ureteral obstruction, remnant kidneys in a 5/6 nephrectomy model, and streptozotocin-induced type I diabetes (95). Recently, a commercially available ELISA has been developed to quantitate OPN in mouse, rat, or human urine, and more studies in rodents and humans are needed to determine whether OPN is an early diagnostic quantitative and sensitive indicator of AKI.

Fatty Acid–Binding Protein

Fatty acid-binding proteins (FABPs) are small (15 kDa) cytoplasmic proteins abundantly expressed in all tissues with active fatty acid metabolism (96). Two types of FABP have been identified in the human kidney: liver-type FABP (L-FABP) in the proximal tubule and heart-type FABP (H-FABP) in the distal tubule (97,98). Free fatty acids (FFAs) in proximal tubules are bound to cytoplasmic FABPs and transported to mitochondria or peroxisomes, where they are metabolized by β -oxidation (99). Urinary L-FABP has been identified in preclinical and clinical models and has been found to be a potential biomarker in a number of pathologic conditions, including chronic kidney disease, diabetic nephropathy, IgA nephropathy, and contrast nephropathy. Using human L-FABP (hL-FABP) transgenic mice, it has been demonstrated that protein-overload nephropathy and unilateral ureteral obstruction, two models of renal interstitial injury, are associated with increased expression and urinary excretion of L-FABP (100,101). In both injury models, attenuation of tubulointerstitial damage was observed in the transgenic mice when compared with wild-type mice, supporting the notion that L-FABP plays a protective role in the setting of increased renal tubular stress (102). In

clinical studies, L-FABP has been advocated as a potential biomarker for monitoring progression of chronic kidney disease (CKD). Kamijo et al. reported increasing L-FABP levels with deterioration of renal function in patients with nondiabetic CKD (101). In addition, Nakamura et al. have reported that urinary L-FABP may serve as a noninvasive biomarker to discriminate between IgA nephropathy and thin basement membrane disease (103) as well as a potential predictive marker for contrast-induced nephropathy (104).

Although L-FABP appears to be an attractive candidate biomarker for a number of renal diseases, additional studies are needed to determine the utility of L-FABP in AKI, especially in the setting of ischemia/reperfusion injury, nephrotoxin exposure, and sepsis.

Sodium/Hydrogen Exchanger Isoform

The sodium/hydrogen exchanger isoform (NHE3) is the most abundant apical sodium transporter in the renal tubule, responsible for the proximal reabsorption of 60%–70% of filtered sodium and bicarbonate in mice (105,106). NHE3 localizes to the apical membrane and intracellular vesicular compartment of renal proximal tubular cells as well as the apical membrane of the thick and thin ascending limb cells (107). McKee et al. demonstrated that NHE3 was readily detected in the urine of healthy rats in immunoblotting experiments (108). Later studies confirmed the presence of NHE3 in urinary exosomes (109). In a study of 68 critically ill adults, du Cheyron et al. performed semiquantitative immunoblotting on urine membrane fractions and found urinary NHE-3 excretion to be a useful marker in discriminating between control patients, those with prerenal azotemia, those with acute glomerular disease, and those with ischemic/nephrotoxic ATN (105). It was recently reported, however, that specimen storage and processing in this study were suboptimal, possibly resulting in increased degradation and decreased recovery of NHE3 prior to protein quantification (109).

Fetuin A

Fetuin-A is an acute phase protein synthesized in the liver and secreted into the circulation, where it has been implicated in several diverse functions, including bone resorption, regulation of insulin activity and hepatocyte growth factor activity, response to inflammation, and inhibition of ectopic mineralization (110). Zhou et al. identified urinary exosomal fetuin-A (EF-A) to be markedly increased in rats following cisplatin injection. Urinary EF-A increased greater than 50-fold at day 2, preceding changes in serum creatinine and histologic evidence of tubule damage by one day, and remained elevated until day 5, when tubule damage was most severe (111). Increased urinary EF-A was additionally noted following I/R injury, but not in the setting of prerenal azotemia (111). In a limited number of clinical specimens (n = 9), urinary EF-A was found to be much higher in ICU patients with AKI compared with ICU patients without AKI and healthy volunteers (111). Immunohistochemical staining localized fetuin-A to the cytoplasm of damaged proximal tubule cells with higher concentrations evident with increasing severity of injury. Although the function of fetuin-A in AKI remains unknown, it may play a role in tubule cell apoptosis.

Significant issues with assay throughput and sensitivity currently complicate the quantification of exosomal-associated proteins, limiting their practical use in large-scale studies of AKI. Published studies have exclusively employed immunoblotting, a labor-intensive and semiquantitative method. In addition, isolation of urinary exosomes requires ultracentrifugation of specimens, a process that takes 1–2 h and several experimental maneuvers. Fortunately, Cheruvanky et al. (112) recently reported that exosomal isolation may be simplified considerably through the use of a commercially available nanomembrane concentrator.

METHODS TO QUANTITATE BIOMARKERS

The traditional method to quantitate urinary enzymes has been enzyme-substrate-based colorimetric assays followed by measurement using a spectrophotometer. As urinary proteins were identified as potential biomarkers of AKI, however, the assay of choice became ELISA, which is based on the detection of an antigen using two epitopically distinct antibodies. There are, however, multiple disadvantages of the ELISA assay: (a) Only one antigen can be detected in one plate; (b) the dynamic range of the assay is usually low, requiring repeat measurements with dilution or concentration of urine samples so that the antigen concentration will fit in to the linear range of the standard curve; (c) the urine sample volume requirement is at least 200 μ l per assay (100 μ l in duplicate); and (d) at least 5–7 h are required to get the results.

A recent technique that is an adaptation of an ELISA, using principles similar to a flow cytometer, is a particle-based flow cytometric assay developed by Luminex® that uses a microfluidics platform incorporating 5.6- μ m beads coupled with the primary (capture) antibody (113). Each microsphere is labeled with a precise ratio of red- and orange-emitting fluorochromes, giving it a unique spectral signature. Classification of each bead is determined with an excitation wavelength of 635 nm and measurement of the emission wavelengths, together with the intensities, of each dye. Quantification is achieved by addition of a biotinylated secondary antibody and streptavidin coupled to a third fluorochrome (phycoerythrin), which is excited at 532 nm using a second laser. The signal is directly proportional to the amount of antigen bound at the microbead surface.

We recently developed a microbead-based assay to measure urinary KIM-1 and NGAL in patients with acute kidney injury and found that the dynamic range was linear up to five orders of magnitude using only 30 μ l of urine, with intra- and interassay variability less than 20% (V. Vaidya and J. Bonventre, unpublished data). Another important advantage of this technique is its multiplexing capability. Theoretically, one is capable of quantitating up to 100 different antigens (owing to the unique spectral signature of each bead) in the same biological sample simultaneously. Researchers have thus far successfully used this technology to quantitate 15–18 different analytes in the same biological sample (113,114). In the urine, this technology has been often used to measure cytokines; however, customized assays have also been developed to quantitate CXCR3-binding chemokines (Mig/CXCL9, IP-10/CXCL10, and ITAC/CXCL11) as indicators of acute renal allograft dysfunction (115). This technique has also been used to measure beta-amyloid, total tau (T-TAU), and hyperphosphorylated tau (P-TAU) in cerebrospinal fluid as biomarkers for Alzheimer's disease (116). Both the ELISA and the microbead-based assay require two epitopically distinct, high-affinity antibodies.

Recently, with advances in nanotechnology, it has been possible to detect antigens using just one capture antibody with a read-out that is based on the principle of a change in conductance (117) owing to antigen binding to the antibody. A silicon nanowire sensor array has been developed that incorporates silicon nanowires covalently coupled with the capture antibody for the respective antigen (117,118). In the process of nanowire fabrication, the conventional gate metal electrode of a field-effect transistor is replaced by the aldehyde-amino covalent bonding of antibodies on top of the silicon oxide layer. The source and drain remain as nickel metal electrodes. In a microfluidic channel, antigen solution flows onto the chip surface, where antigens bind to the respective monoclonal antibodies covalently coupled to the nanowires. Depending on the charge of the antigen [positive (p-type) or negative n-type)], carriers in the channel between source and drain increase or decrease (depending on if they are p-type or n-type, respectively), and therefore increase or decrease the conductance of the transistor (119). The conductance is easily measurable and a change in conductance is directly related to the amount of antigens bound. Such a multiplexed electrical detection nanowire sensor array was used to detect prostate-specific antigen (PSA), PSA- α 1-antichymotrypsin, carcinoembryonic

antigen, and mucin-1 in serum for the diagnosis of prostate cancer (118). This technology is in its initial stages and requires additional evaluation and validation, but this general approach offers significant advantages over the existing technology: (a) its sensitivity is in the femtomolar range, (b) it requires only one antibody as opposed to two epitopically distinct antibodies; (c) it produces a read-out within minutes as opposed to hours; and (d) it can be adapted as a bedside technology for patient care in hospitals.

CONCLUSIONS

AKI is a common and devastating condition associated with significant morbidity and mortality. Efforts to identify biomarkers to assist with the early diagnosis of AKI have yielded many promising candidates, such as KIM-1, NGAL, IL-18, Cys-C, clusterin, FABP, and osteopontin. A single biomarker may not be adequate to define AKI given inherent renal heterogeneity and the disparate settings under which kidney injury occurs (120,121). Qualification of biomarkers will require large, well-designed prospective studies comparing multiple biomarkers in the same set of urine samples over extended time courses. Such studies will allow temporal patterns of biomarker elevation to be established, patterns that may be specific to the mechanism of injury (nephrotoxicant, ischemia, allograft rejection, etc.), population of interest (elderly, pediatric, etc.), and/or co-occurring disease states (diabetes, heart disease, sepsis, etc.). As the utility of a biomarker or biomarker panel emerges for the detection of AKI, considerable effort will need to be directed toward developing technologies that will permit the rapid detection and quantification necessary in clinical practice. Biomarkers have the potential to transform the way we diagnose and treat patients with AKI.

SUMMARY POINTS

1. Acute kidney injury is a complex condition and over the past 50 years, mortality rates have remained essentially unchanged at approximately 50%–70%.
2. The pathophysiological mechanisms that can contribute to AKI due to toxins or changes in renal hemodynamics include alterations in renal perfusion resulting from loss of autoregulation and increased renal vasoconstriction; tubular dysfunction resulting from structural changes, metabolic alterations, loss of cell polarity, cell death; abnormalities of tubuloglomerular balance; and a proinflammatory milieu further compromising microvascular perfusion and decline of oxygen and nutrients to the tubules. There is a very delicate and dynamic relationship between renal injury and tissue repair, which governs the progression or regression of renal injury that determines the ultimate outcome.
3. Traditional blood (creatinine, blood urea nitrogen) and urine markers of kidney injury (epithelial cells, tubular casts, fractional excretion of Na^+ , urinary concentrating ability, etc.) are insensitive and nonspecific for the diagnosis of AKI.
4. To date, most studies have emphasized discovery, characterization, and validation of several highly promising individual biomarkers using models of kidney injury in animals or varied states in humans. Future research should be aimed at evaluating and validating these biomarkers individually and in a multiplexed manner to define (a) preclinical and clinical sensitivity and specificity; (b) time course of elevation in the urine after kidney damage; (c) the correlation with renal tubular damage; and (d) predictions of long and short term outcomes. At a practical level, it is important to define urine preservation techniques and characteristics of the urine that interfere with the measurement of each biomarker. Finally, optimal use of biomarkers requires quantitative algorithms that will take advantage of multiple biomarkers to predict onset of AKI and its outcome and facilitate timely intervention.

5. Future research should also be directed toward developing and validating high-throughput technologies for biomarker quantitation, with an ultimate goal of having a bedside technology to provide online noninvasive detection of the onset and severity of kidney injury.

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a Pathophysiology of AKI

- Vasoconstriction
- Desquamation of tubular cells
- Intraluminal tubular obstruction resulting in tubular backleak
- Local production of inflammatory mediators resulting in interstitial inflammation, small vessel obstruction, and local ischemia

b Cellular level

Figure 1. Schematic representation of (a) pathophysiological and (b) cellular mechanisms of acute kidney injury.

a Kidney injury continuum **b** Biomarkers **Figure 2.**

(a) **Kidney injury continuum:** The process of acute kidney injury can be divided into various reversible stages depending on the severity of insult, starting from increased risk to damage followed by decrease in glomerular filtration rate (GFR) further progressing to kidney failure and death. Diminished renal perfusion may result in decreased GFR in the absence of intrinsic damage, a condition termed prerenal azotemia. (b) **Biomarkers of AKI:** Traditionally used markers, such as blood urea nitrogen (BUN) and creatinine (CR), are insensitive, nonspecific, and do not adequately differentiate between the different stages of AKI. A delay in diagnosis prevents timely patient management decisions, including administration of putative therapeutic

agents. Urinary biomarkers of AKI will facilitate earlier diagnosis and specific preventative and therapeutic strategies, ultimately resulting in fewer complications and improved outcomes.

Table 1

Proposed classification scheme for acute kidney injury (AKI) in patients*

Stage	Creatinine criteria	Urine output criteria
1 (Risk)	Increased SCr of $\geq 0.3 \text{ mg dl}^{-1}$ or increase to $\geq 150\%$ – 200% from baseline	UO $< 0.5 \text{ ml kg}^{-1} \text{ h}^{-1}$ for $> 6 \text{ h}$
2 (Injury)	Increased SCr to $> 200\%$ – 300%	UO $< 0.5 \text{ ml kg}^{-1} \text{ h}^{-1}$ for $> 12 \text{ h}$
3 (Failure)	Increase SCr to $> 300\%$ from baseline (or SCr $\geq 4 \text{ mg dl}^{-1}$) (acute rise $\geq 0.5 \text{ mg/dl}$)	UO $< 0.3 \text{ ml kg}^{-1} \text{ h}^{-1} \times 24 \text{ h}$ or Anuria $\times 12 \text{ h}$

*Stages eliminated from the original RIFLE criterion*Loss Persistent ARF = complete loss of kidney function $> 4 \text{ weeks}$ ESKD End-stage kidney disease ($> 3 \text{ months}$)

ARF: Acute renal failure; ESKD: end-stage kidney disease; RIFLE: Risk of renal dysfunction; injury to the kidney; failure of kidney function; loss of kidney function; end-stage kidney disease; SCr: serum creatinine; UO: urine output.

* Adapted from Reference 23.

Table 2**Biomarkers of acute kidney injury**

Biomarker	Comments	Detection	Species
Alanine aminopeptidase (AAP)	1. Proximal tubule brush border enzyme 2. Instability may limit clinical utility	Colorimetry	Rat, dog, human
Alkaline phosphatase (AP)	1. Proximal tubule brush border enzyme. Human intestinal alkaline phosphatase is specific for proximal tubular S3 segment; human tissue nonspecific alkaline phosphatase is specific for S1 and S2 segments 2. Levels may not correlate with extent of functional injury 3. Instability may limit clinical utility	Colorimetry	Rat, human
α -glutathione-S-transferase (α -GST)	1. Proximal tubule cytosolic enzyme 2. Requires stabilization buffer for specimen storage and processing 3. Upregulated in AKI and renal cell carcinoma	ELISA	Mouse, rat, human
γ -glutamyl transpeptidase (γ GT)	1. Proximal tubule brush border enzyme 2. Instability requires samples to be analyzed quickly after collection, limiting clinical utility	Colorimetry	Rat, human
N-acetyl- β -glucosaminidase (NAG)	1. Proximal tubule lysosomal enzyme 2. More stable than other urinary enzymes 3. Extensive preclinical and clinical data in a variety of conditions (nephrotoxicant exposure, cardiopulmonary bypass, delayed renal allograft function, etc.) 4. Endogenous urea may inhibit activity	Colorimetry	Mouse, rat, human
β_2 -microglobulin	1. Light chain of the MHC I molecule expressed on the cell surface of all nucleated cells 2. Monomeric form is filtered by the glomerulus and reabsorbed by the proximal tubule cells 3. Early marker of tubular dysfunction in a variety of conditions	ELISA, nephelometer	Mouse, rat, human

Biomarker	Comments	Detection	Species
	4. Instability in acidic urine limits clinical utility		
α_1 -microglobulin	1. Synthesized by the liver 2. Filtered by the glomerulus and reabsorbed by proximal tubule cells 3. Early marker of tubular dysfunction; high levels may predict poorer outcome 4. Stable across physiologic urinary pH	ELISA, nephelometer	Mouse, rat, human
Retinol-binding protein (RBP)	1. Synthesized by liver, involved in vitamin A transport 2. Filtered by glomerulus and reabsorbed by proximal tubule cells 3. Early marker of tubular dysfunction 4. Increased stability in acidic urine when compared to β_2 -microglobulin	ELISA, nephelometer	Mouse, rat, human
Cystatin C	1. Important extracellular inhibitor of cysteine proteases 2. Filtered by the glomerulus and reabsorbed by proximal tubule cells 3. Elevated urinary levels reflect tubular dysfunction; high levels may predict poorer outcome	ELISA, nephelometer	Mouse, rat, human
Microalbumin	1. Established marker for monitoring progression of diabetic kidney disease 2. Elevated urinary levels may be indicative of proximal tubular damage 3. Lack of specificity for AKI may limit its utility	ELISA Immunoturbidimetry	Mouse, rat, dog, monkey, human
Kidney injury molecule-1 (KIM-1)	1. Type-1 cell membrane glycoprotein upregulated in dedifferentiated proximal tubule epithelial cells 2. Ectodomain is shed and can be quantitated in urine following acute kidney injury in preclinical and clinical studies 3. Elevated urinary levels are highly sensitive and specific for AKI 4. Upregulated following various models of preclinical and clinical AKI, fibrosis,	ELISA, Luminex®-based assay	Zebrafish, mouse, rat, dog, monkey, human

Biomarker	Comments	Detection	Species
Clusterin	<p>renal cell carcinoma, and polycystic kidney disease</p> <ol style="list-style-type: none"> 1. Expressed on dedifferentiated tubular epithelial cells 2. Elevated kidney and urinary levels are very sensitive for AKI in preclinical models 3. Upregulated in various rodent models of AKI, fibrosis, renal cell carcinoma, and polycystic kidney disease 4. No clinical study demonstrating its use 	ELISA	Mouse, rat, dog, monkey, human
Neutrophil gelatinase associated lipocalin (NGAL)	<ol style="list-style-type: none"> 1. Initially identified bound to gelatinase in specific granules of the neutrophils but also may be induced in epithelial cells in the setting of inflammation or malignancy 2. Expression upregulated in kidney proximal tubule cells and urine following ischemic or cisplatin induced renal injury 3. Found to be an early indicator of AKI following cardiopulmonary bypass 4. Specificity for AKI in setting of sepsis; pyuria needs to be further established 	ELISA Luminex®-based assay	Mouse, rat, human
Interleukin-18 (IL-18)	<ol style="list-style-type: none"> 1. Cytokine with broad immunomodulatory properties, particularly in setting of ischemic injury 2. Constitutively expressed in distal tubules; strong immunore-activity in proximal tubules with transplant rejection 3. Elevated urinary levels found to be early marker of AKI and independent predictor of mortality in critically ill patients 	ELISA Luminex®-based assay	Mouse, rat, human
Cysteine-rich protein (CYR-61)	<ol style="list-style-type: none"> 1. Induced in proximal straight tubules of kidney and secreted in the urine within 3–6 h following ischemic kidney injury 2. Urinary levels decrease rapidly in spite of progression of injury indicating stability issue 3. No clinical study demonstrating its use 4. No quantitative method established 	Western blot	Mouse, rat, human

Biomarker	Comments	Detection	Species
Osteopontin (OPN)	<ol style="list-style-type: none"> 1. Upregulated in various rodent models of AKI 2. The induction correlates with inflammation and tubulointerstitial fibrosis 3. No clinical study demonstrating its use 	ELISA	Mouse, rat, monkey, human
Fatty acid binding protein (FABP)	<ol style="list-style-type: none"> 1. Expressed in proximal tubule epithelial cells 2. Current evidence suggests clinical utility as a biomarker in CKD and diabetic nephropathy 3. Additional studies necessary to determine utility in setting of preclinical and clinical AKI 	ELISA	Mouse, rat, human
Sodium/hydrogen exchanger isoform (NHE3)	<ol style="list-style-type: none"> 1. Most abundant sodium transporter in the renal tubule 2. Urinary levels found to discriminate between prerenal azotemia and AKI in ICU patients 3. Samples require considerable processing, limiting assay throughput 	Immunoblotting	Mouse, rat, human
Exosomal fetuin-A	<ol style="list-style-type: none"> 1. Acute phase protein synthesized in the liver and secreted into the circulation 2. Levels in proximal tubule cell cytoplasm correspond to degree of injury 3. Urinary levels found to be much higher in ICU patients with AKI compared to ICU patients without AKI and healthy volunteers 4. Samples require considerable processing, limiting assay throughput 5. Additional studies necessary to determine utility in setting of preclinical and clinical AKI 	Immunoblotting	Rat, human

Table 3**Characteristics of Kidney injury molecule-1 (Kim-1)***

Parameters	Description	
Other names	Hepatitis A virus cellular receptor-1 (HAVCR-1), T cell immunoglobulin like molecule-1 (TIM-1), cochlear injury molecule-1 (CIM-1)	
Chromosomal location	Human (5q31), chimpanzee (5), rat (10q21), mouse (11B1.1)	
Molecular mass	Human (359 aa), chimpanzee (predicted: 357 aa), rat (307 aa), mouse (305 aa)	
Structure	Immunoglobulin domain, threonine-serine-proline-rich mucin domain, transmembrane domain, short cytoplasmic tail	
Number of KIM-1 homologues	Human (three: KIM-1, KIM-3, KIM-4), rat (six: Kim-1 to Kim-6), mouse (eight: Kim-1 to Kim-8)	
% Identity of human KIM-1 protein	Chimpanzee (79%), rat (39%), mouse (37%)	
Upregulation in kidney injury/toxicity/dedifferentiation models	Adriamycin-nephrosis	Ischemia/reperfusion
	Brain dead donor rat kidney	Kidney fibrosis
	Cadmium	Mercuric chloride
	Chromium	Ochratoxin
	Cisplatin	Polycystic kidney disease
	Cyclosporine	Protein-overload
	Endotoxin	Renal cell carcinoma
	Folic acid	Sevoflurane
Gentamicin	S-(1,1,2,2-tetrafluoroethyl)-l-cysteine (TFEC)	

* Adapted and modified from Vaidya and Bonventre, 2006 (27).