

Biomarkers for the Diagnosis of Acute Kidney Injury

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Key Words

Acute kidney injury · Acute renal failure · Biomarker · Diagnosis

Abstract

The identification of acute kidney injury relies on tests like blood urea nitrogen and serum creatinine that were identified and incorporated into clinical practice several decades ago. This review summarizes clinical studies of newer biomarkers that may permit earlier and more accurate identification of acute kidney injury. The urine may contain sensitive and specific markers of kidney injury that are present due to either impaired tubular reabsorption and catabolism of filtered molecules or release of tubular cell proteins in response to ischemic or nephrotoxic injury. Many potential markers have been studied. Promising injury markers in the urine include N-acetyl- β -D-glucosaminidase, neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, and interleukin-18. New biomarkers of kidney injury hold the promise of substantially improving the diagnostic approach to acute kidney injury. Adequately powered clinical studies of multiple biomarkers are needed to qualify the biomarkers before they can be fully adopted in clinical practice. Once adopted, more sensitive biomarkers of acute kidney injury hold the potential to transform the care of patients with renal disease.

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Introduction

Acute renal failure, now termed acute kidney injury (AKI), is an increasingly common and devastating complication in hospitalized patients. The current diagnostic

tests for AKI include serum creatinine (SCr) and blood urea nitrogen (BUN), two biomarkers that were identified and incorporated into clinical practice several decades ago. It is now widely appreciated that SCr and BUN are suboptimal markers for AKI, and that more sensitive, specific, and early biomarkers are needed. This review sets out to cover recent developments in the field of AKI biomarker validation in clinical studies.

Injury versus Failure: Towards a Troponin for the Kidney

The new term 'acute kidney injury' instead of 'acute renal failure' – by its replacement of the word 'injury' for 'failure' – hints towards a paradigm shift in nephrology. The diagnosis of 'failure' refers to the kidney's inability to perform one of its major functions, namely glomerular filtration. The diagnosis of glomerular filtration failure is made only after endogenous filtration markers (BUN or SCr) have accumulated in the blood, typically hours or even days after an inciting event. The diagnosis of 'injury', by contrast, does not presuppose a reduction in glomerular filtration. Newer biomarkers are needed to identify correlates of cellular (typically tubular) injury, which may be present well before or in the absence of a reduction in GFR. The analogy to cardiology may be instructive: clinicians diagnosing acute myocardial infarction do not wait until a reduction in cardiac output, but rather make the diagnosis of myocardial injury on the basis of elevations of tissue-specific biomarkers in the serum.

The biological response of kidney tissue to ischemic or nephrotoxic injury may be utilized as early indicators of

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AKI. Urine is readily accessible and has been proposed to be a more promising biological fluid than serum or plasma to identify the earliest markers of kidney injury. Time honored tests like urinary microscopy, urine osmolality, and fractional excretion of sodium or urea are nonspecific and insensitive, although careful and large prospective studies have not been conducted [1]. The urine may contain more sensitive and specific markers of kidney injury that are present due to impaired tubular reabsorption and catabolism of filtered molecules, release of enzymes or exosomes from tubular cells, and as a response of tubular cells to ischemic or nephrotoxic injury. This review will focus on several promising urinary biomarkers under investigation.

Tubular Enzymes and Markers of Tubular Dysfunction

The apical surface of proximal tubular epithelial cells contains numerous microvilli that form the brush border and contain proteins with enzymatic functions to carry out the specialized tasks of the proximal tubule. Intracellular enzymes can be released into the urine with injury [2] either by exocytosis or leakage. The proteins can exist in the free form or may be membrane-encased as exosomes. Several different classes of enzymes can be found: lysosomal proteins, such as N-acetyl- β -D-glucosaminidase (NAG), brush border enzymes including gamma-glutamyl transferase (GGT) and alkaline phosphatase, as well as cytosolic proteins such as α -glutathione S-transferase (α -GST). Furthermore, when proximal tubular epithelial cells are injured, they may not metabolize cystatin C properly, and filtered intact cystatin C may appear in the urine. Similarly, injured cells may not completely reabsorb low-molecular-weight proteins that are freely filtered into the urinary space, such as α_1 - and β_2 -microglobulin.

Westhuyzen et al. [3] compared the predictive value of a number of tubular enzymes for the subsequent development of AKI, defined as a 50% rise in serum creatinine to at least 1.7 mg/dl. Four of 26 subjects developed AKI; baseline levels of GGT, AP, NAG, α -GST and π -GST were higher in those who developed AKI, compared to those who did not. α -GGT and π -GST had the best predictive value on their own, with areas under the receiver-operating characteristic curve (AUC-ROC) of 0.95 (95% CI 0.79–1.0) and 0.93 (95% CI 0.74–1.0), respectively. Changes in enzyme levels preceded detectable changes in timed creatinine clearance. However, when the authors attempted to develop cutpoints based on this small study and tested the generalizability of their results in a test population of 19 patients (4 of whom developed AKI), the sensitivity and specificity of these biomarkers were significantly reduced.

Several investigators have examined the ability of tubular enzymes to predict adverse clinical outcomes. Herget-Rosenthal et al. [4] risk-stratified patients with non-oliguric AKI (defined as a doubling in creatinine from a baseline concentration of $<106 \mu\text{mol/l}$ to at least $115 \mu\text{mol/l}$) using tubular enzymes as biomarkers. They identified 73 subjects who met prespecified criteria for AKI; 26 of these individuals subsequently required dialysis. They measured urinary excretion of cystatin C, α_1 - and β_2 -microglobulin, α -GST, NAG, retinol-binding protein (RBP), GGT and lactate dehydrogenase on the day of study enrollment. Cystatin C and α_1 -microglobulin (markers of abnormal proximal tubule function) had the best predictive value for the need for dialysis, with AUC-ROC curves of 0.92 and 0.86, respectively. Of the tubular enzymes studied, NAG had the best predictive value, with an AUC-ROC of 0.81. In another study, Chew et al. [5] found that levels of NAG and tissue non-specific alkaline phosphatase were higher in AKI patients with poor outcomes (defined as need for dialysis or death). Liangos et al. [6] recently performed a study of NAG and kidney injury molecule-1 (KIM-1, a tubular injury marker, discussed below) in 201 patients with established AKI. They found that elevated NAG levels portended poor clinical outcomes, with the odds of death or dialysis requirement increased over five-fold in patients with the highest versus lowest quartiles or urinary NAG levels, even after careful multivariable adjustment for disease severity and comorbidity. The predictive power of KIM-1 levels in this study will be discussed below.

Tubular enzymes present in the urine have long been studied as markers of AKI, but they have not been adopted in widespread clinical use either as early diagnostic tests, prognostic indicators, or surrogate endpoints for interventional studies. Some authors have suggested that tubular enzymes are overly sensitive, because they tend to rise after injuries such as cardiopulmonary bypass without an associated rise in SCr [7, 8]. Investigators should exercise caution, however, in interpreting performance characteristics of new biomarkers against a gold standard like SCr that has poor specificity and sensitivity: cardiac troponin would appear to be nonspecific against earlier (and now discredited) cardiac biomarkers like lactate dehydrogenase.

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

NGAL is one of the best-studied urinary biomarkers of AKI to date. Also known as lipocalin-2 or siderocalin, NGAL was first discovered as a protein in granules of hu-

man neutrophils; animal studies showed its promise as an early marker of ischemic and nephrotoxic kidney injury [9].

Mishra et al. [10] prospectively obtained serial urine and serum samples from 71 children undergoing cardiopulmonary bypass for surgical correction of congenital heart disease. Twenty children developed AKI, defined as a 50% increase in SCr. Urinary NGAL at just 2 h following CPB almost perfectly predicted which patients would go on to develop AKI (AUC-ROC 0.998). Serum NGAL was inferior to urinary NGAL for the identification of AKI. As encouraging as these results were, it should be noted that 29% of eligible patients were excluded due to perioperative use of ibuprofen, angiotensin-converting enzyme inhibitors, gentamicin, or vancomycin. A larger follow-up study of 120 children (using similar exclusion criteria) by Dent et al. [11] showed that 2-hours postoperative serum NGAL was predictive of AKI (AUC-ROC 0.96) and correlated with postoperative change in SCr, duration of AKI, and length of stay.

Wagner reported less sanguine results on urinary NGAL in 81 adult patients undergoing cardiac surgery; the only exclusion criterion was pre-existing end-stage renal disease [12]. Sixteen patients developed AKI, defined as a 50% increase in SCr. Urinary NGAL levels were consistently higher immediately postoperatively and at 1, 3, 18, and 24 h postoperatively in patients who went on to develop AKI. Substantial overlap, however, was noted between patients who did and did not develop AKI. The AUC-ROC for NGAL ranged from 0.67 (immediately after surgery) to 0.80 (18 h following surgery). The salient differences between the pediatric and adult cardiac surgery cohorts included patient age, comorbidity, and exclusion criteria.

Parikh et al. [13] studied urinary NGAL in 53 consecutive patients undergoing living or deceased donor kidney transplantation. NGAL levels (normalized to urine creatinine concentration) were significantly higher in deceased donor recipients with delayed graft function (DGF) (n = 10, median 3,306 ng/mg creatinine) than prompt graft function (n = 20, median 756 ng/mg creatinine). A cutoff value of 1,000 ng/mg creatinine had 90% sensitivity and 83% specificity for the identification of DGF; the AUC-ROC was 0.90.

Zappitelli et al. [15] studied urinary NGAL in 140 children admitted to the intensive care unit requiring mechanical ventilation. Urine was collected daily for 4 days. The authors found on cross-sectional analysis that mean and peak urinary NGAL levels were higher in patients with worsening degrees of AKI (as judged by the pediatric RIFLE criteria). At 48 h prior to the development of AKI,

urinary NGAL had an AUC-ROC of 0.79 for the subsequent development of AKI.

Urinary NGAL has also been studied as a surrogate marker of kidney injury from aprotinin, a nephrotoxic fibrinolytic used in cardiac surgery. Wagner et al. [36] found that postoperative levels of urinary NGAL were almost 20 times higher in patients who received aprotinin compared to epsilon amino-caproic acid, lending support to the suggestion of aprotinin's nephrotoxicity. Urinary NGAL was also found to be higher after coronary angiography in 13 patients with AKI than in 27 controls without AKI [17].

Interleukin-18

IL-18 was found to potentiate ischemic AKI and to be detectable in the urine of mice subjected to ischemic kidney injury [18]. Urinary IL-18 has been studied by Parikh and coworkers in a variety of clinical settings, including delayed graft function [14], cardiac surgery [19], acute respiratory distress syndrome [20], and cross-sectionally in patients with and without kidney disease [21].

The first AKI study of urinary IL-18 in humans was a cross-sectional comparison of patients with ATN (n = 14), pre-renal azotemia (n = 8), UTI (n = 5), CKD (n = 12), transplant recipients (n = 22), and healthy controls (n = 11) [21]. The highest levels of urinary IL-18 were observed in patients with ATN and delayed graft function, with relatively little overlap from patients with pre-renal azotemia, urinary tract infections, and CKD. The AUC-ROC from this cross-sectional cohort (for the identification of ATN, including delayed graft function) was 0.95, with a sensitivity of 85% and specificity of 88% at a cutoff of 500 pg IL-18/mg creatinine.

The NIH sponsored Acute Respiratory Distress Syndrome Network trial of low versus high tidal volume ventilation was the source of urine samples in a subsequent study of urinary IL-18 [20]. Parikh and colleagues performed a prospective, nested, case-control study in 138 of the 861 patients enrolled; exclusion criteria included a baseline SCr >1.2 mg/dl. They found that urinary IL-18 levels were higher in those patients who developed AKI (defined as a 50% increase in SCr within 6 days of enrollment), and that higher levels were predictive of mortality. The AUC-ROC for IL-18 (not normalized to urine creatinine) was 0.73 at 24 h prior to AKI diagnosis; this value does not compare favorably with the AUC-ROC of 0.95 from the cross-sectional study of urinary IL-18.

Parikh et al. [20] also measured IL-18 in urine samples collected in the pediatric cardiac surgery cohort used to

study NGAL. They measured IL-18 in all 20 cases of AKI and in 35 of the 51 non-AKI cases (matched according to race, gender, and age to AKI cases). Compared to NGAL, which increased 25-fold within 2 h and declined after 6 h of CPB, IL-18 increased at 4–6 h and remained elevated up to 48 h following CPB. The reported AUC-ROCs for IL-18 were 0.61 at 4 h, 0.75 at 12 h, and 0.73 at 24 h, substantially lower than the 0.998 reported by Mishra for NGAL at 2 h following CPB.

IL-18 was also studied by Washburn et al. [14] in critically ill children requiring mechanical ventilation (identical cohort as studied by Zappitelli et al. [15] for NGAL). They found on cross-sectional analysis that peak urinary IL-18 levels were higher in patients with worsening degrees of AKI (as judged by the pediatric RIFLE criteria). However, in prospective analysis IL-18 demonstrated no ability to predict the subsequent development of AKI (AUC-ROC 0.54). Not surprisingly for a pro-inflammatory cytokine that plays an important role in sepsis, urinary IL-18 was significantly higher in patients with sepsis than in those without, and limited its diagnostic ability for the early identification of AKI in this cohort.

Urinary IL-18 has also been studied as a biomarker of contrast nephropathy with mixed results. Ling et al. [17] found higher urinary levels in 13 patients who developed AKI following coronary angiography than in 27 non-AKI controls ($p < 0.01$), whereas Bulent et al. [22] found no difference in a similar number of patients with and without AKI.

Na⁺/H⁺ Exchanger Isoform 3 (NHE3)

NHE3 is the most abundant sodium transporter in the renal tubule and is responsible for proximal reabsorption of up to 70% of filtered sodium and bicarbonate. Du Cheyron et al. [23] performed a cross-sectional study of 68 patients admitted to the ICU. They isolated membrane fractions from the urine and measured NHE3 concentrations using semiquantitative immunoblotting. NHE3 protein was undetectable in patients without AKI ($n = 14$), detectable at relatively low levels in patients with pre-renal azotemia ($n = 17$) and post-renal obstruction ($n = 3$), and was significantly elevated in ATN ($n = 17$; 6.6-fold higher than in pre-renal azotemia). The same investigators also measured urinary retinol-binding protein (RBP), the primary plasma transport protein for vitamin A which is filtered by the glomerulus and then reabsorbed by the proximal tubule. Urinary RBP was significantly higher in patients with ATN than normal controls, but significant overlap was noted, particularly with pre-renal azotemia.

Kidney Injury Molecule-1 (KIM-1)

Kidney injury molecule-1 ('KIM-1' in humans, or 'Kim-1' in rodents) was identified as the single most upregulated gene in postischemic rat kidney using a PCR-based technique [24]. *KIM-1* encodes a type I cell membrane glycoprotein containing, in its extracellular portion, a novel six-cysteine immunoglobulin-like domain and a threonine/serine and proline-rich domain characteristic of mucin-like O-glycosylated proteins, suggesting its potential involvement in cell-cell and/or cell-matrix interactions [25]. After proximal tubular kidney injury, the ectodomain of KIM-1 protein is shed from cells into the urine in rodents and in humans. In both ischemia-reperfusion and cisplatin-induced nephrotoxicity models in the rat, urinary Kim-1 is a sensitive and specific indicator of proximal tubular kidney injury and is increased earlier than any of the conventional biomarkers, e.g. plasma creatinine, blood urea nitrogen, glycosuria, proteinuria, and urinary NAG [26]. In recently completed studies of 8 mechanistically different proximal tubule nephrotoxicants and 2 different hepatotoxicants in rats, Kim-1 had an AUC-ROC of 0.99 for proximal tubular toxicity, using histopathology as the gold standard; of 21 urinary markers studied, Kim-1 was found to be to have the highest sensitivity and specificity [27].

Human studies have begun to confirm the promise of urinary KIM-1 for the diagnosis of AKI. Han et al. [28] demonstrated marked expression of KIM-1 in kidney biopsy specimens from 6 patients with acute tubular necrosis (ATN), and found elevated urinary levels of KIM-1 in 7 patients with ischemic ATN; urinary levels of KIM-1 were significantly lower in contrast nephropathy ($n = 7$), although the levels did correlate with severity of contrast-induced injury. Levels of urinary KIM-1 were lower in AKI not due to ATN ($n = 9$), CKD ($n = 9$), and were below limits of detection in normal subjects ($n = 8$) [28]. KIM-1 is also highly expressed in the setting of renal cell carcinoma (RCC), as shown by Han et al. [29]. KIM-1 was detectable in the urine of patients with RCC, suggesting a potential role for early non-invasive diagnosis. Lin et al. [30] studied KIM-1 protein expression in a large series of 480 neoplasms and found KIM-1 expression in papillary, clear cell, and metastatic RCC but not chromophobe RCC. They also demonstrated KIM-1 staining in 15 of 16 cases of clear cell carcinoma of the ovary. Van Timmeren et al. [31] stained for KIM-1 protein in tissue specimens from 102 patients who underwent kidney biopsy for a variety of kidney diseases and 7 patients who underwent nephrectomy for renal cell carcinoma. No tissue KIM-1 was found in patients with minimal change disease or in the

tumor-free samples of renal cell carcinoma. In all other disease conditions, KIM-1 protein was identified in de-differentiated proximal tubular cells and correlated with tubulointerstitial fibrosis and inflammation. In the subset of patients who underwent urine collection near the time of biopsy, urinary KIM-1 levels correlated with tissue expression of KIM-1. Urinary KIM-1 may therefore hold promise as a non-invasive assessment of the activity and prognosis of a variety of acute and chronic kidney diseases. The diagnostic and prognostic role of KIM-1 in kidney transplant recipients was evaluated by Zhang et al. [32] in 25 protocol biopsies, 25 biopsies of patients with active tubular injury, and 12 patients with acute cellular rejection. Focal KIM-1 expression was found in 28% of protocol biopsies despite the absence of conventional histologic evidence of tubular cell injury. Proximal tubule KIM-1 expression was found in all patients with histologic evidence of tubular cell injury, and higher KIM-1 staining correlated with improved renal outcomes at 18 months. Van Timmeren et al. [33] studied 24-hour urinary KIM-1 excretion in 145 stable renal transplant recipients who were subsequently followed for 4 years. Higher KIM-1 excretion was associated with significantly higher risk of graft loss over the follow-up period. High KIM-1 excretion was also associated with proteinuria, low creatinine clearance, and high donor age, but was independently associated with graft loss after multivariate adjustment for these variables.

Liangos et al. [6] studied urinary KIM-1 (and NAG, see above) at the time of nephrology consultation in 201 patients with established AKI. Because non-AKI controls were not included in this study, diagnostic performance characteristics like sensitivity, specificity, or the area under the receiver operating characteristics curve were not reported. KIM-1 demonstrated prognostic significance: elevated levels were significantly associated with the clinical composite endpoint of death or dialysis requirement, even after adjustment for disease severity or comorbidity. How KIM-1 and other markers will compare to other predictive markers and clinical scoring systems is the subject of extensive ongoing studies.

Other Markers

Keratinocyte-derived chemokine was found by Molls et al. [34] in a mouse model of renal ischemia-reperfusion injury to be elevated in serum and urine 3 h following injury. These investigators measured urinary levels of a structurally homologous molecule in humans, termed human growth-related oncogene- α (Gro- α), in a small

pilot study of patients ($n = 17$) undergoing kidney transplantation, and found markedly higher levels among those with DGF following deceased donor transplantation. Zhou et al. [35] have focused on urinary exosomes (small excreted vesicles which contain membrane and cytosolic proteins) as a potential source of novel AKI biomarkers. Exosomal fetuin-A was identified in preclinical rodent models of ischemic and cisplatin-induced AKI; a small pilot study in 9 humans showed exosomal fetuin-A to be present in the urine of ICU patients with AKI but not in healthy volunteers or ICU patients without AKI [35].

Conclusion

New biomarkers under clinical investigation will likely perform differently with respect to disease specificity (e.g. sepsis vs. nephrotoxic versus postoperative AKI), time course (early vs. late markers), and prognostic characteristics (markers of incipient AKI vs. markers of prognosis in established AKI). Establishing the optimal test(s) for a given clinical scenario will require prospective validation in large numbers of patients with a variety of causes of AKI, preferably with measurement of numerous candidate biomarkers for the purpose of efficiency. The possibility that new biomarkers may be superior to SCr for the identification of AKI will require investigators to test the creatinine-independent associations between biomarker levels and exposures (e.g. cardiopulmonary bypass time, dose of nephrotoxin administration) and outcomes (e.g. not only AKI as defined by creatinine but also length of stay, need for dialysis, and mortality). Early and accurate diagnosis of AKI will allow interventional studies to be performed in a timely fashion, which is a prerequisite for the future development of effective prevention and therapeutic strategies that have eluded nephrology for years.

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