Kidney Injury Molecule-1 (KIM-1) as an early detection tool for acute kidney injury and other renal diseases.

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Abstract

Introduction: Although serum creatinine is the standard metric tool for the detection of renal injury, its lack of sensitivity has made the early diagnosis of acute kidney injury (AKI) very difficult. In fact, the absence of sensitive AKI biomarkers has impaired progress in the nephrology field and had a detrimental effect on the design and outcome of AKI clinical trials. Recently, several proteins have shown potential in the early detection of acute and chronic kidney injuries.

Areas covered: This review discusses the current status of kidney injury molecule-1 (KIM-1) as a potential diagnostic tool in patients with various acute and chronic kidney diseases. The focus is limited to human studies from January 2002 to July 2010. The review clarifies the clinical conditions for which KIM-1 has the greatest potential utility for early detection of kidney injury. It also demonstrates to the reader the barriers to the successful use of KIM-1 and other biomarkers in clinical practice, and the future trials that will be needed to validate their use.

Expert opinion: Despite the early promise of biomarkers such as KIM-1 for the early detection and prognosis of kidney disease, more studies are required to establish their utility in clinical practice. Indeed, the published clinical studies of urine KIM-1 so far are small and insufficient to support clinical studies of urine KIM-1 as an effective AKI diagnostic test in humans. It is suggested, through the heterogeneity of AKI and existing published data, that more than one biomarker may be necessary to obtain sufficient sensitivity and specificity for AKI screening.

Keywords: acute kidney injury, biomarker, early detection, KIM-1, proteinuria

Article Highlight Box

- KIM-1 is a urinary biomarker for acute tubular injury in pediatric and adult patients. Its continuing evaluation in early detection and severity of AKI will require larger prospective studies to establish a cutoff value that is predictive of AKI as well as its temporal expression patterns.

- In the kidney transplant population, tissue KIM-1 is sensitive for kidney injury but not specific for acute rejection. Urinary KIM-1 did not perform better than traditional markers in predicting chronic allograft failure or delayed graft function.

- Further studies to confirm the association between KIM-1 and subtypes of RCC may lead to a non-invasive means of early detection of RCC.

- Conflicting data regarding the association between KIM-1 and proteinuria leave the clinical use of KIM-1 in the CKD population in question.

1] Introduction

1.1 Acute kidney injury

Acute kidney injury (AKI) is an important cause of morbidity and mortality in hospitalized patients. One or more events of ischemic insult and nephrotoxic injury to the kidney are the main cause of hospital acquired AKI. The incidence of hospital acquired AKI varies from 5% in patients with normal renal function to 25% in intensive care unit (ICU) patients [1-3]. Mortality rates of patients with postoperative AKI range from 50 to 70% among ICU patients who require renal replacement therapy (RRT) [4, 5]. For the last 30 years, the mortality rate of patients with

severe AKI requiring RRT in the ICU has not decreased significantly despite advances in supportive care, including continuous RRT.

1.2 Limitation of current AKI criteria

A standard definition of AKI is lacking, with > 30 definitions used for diagnosis of AKI in published studies, most based on serum creatinine (Scr) values. Two new definitions of AKI have been developed recently: RIFLE (risk, injury, failure, loss, and end stage renal disease) and AKIN (acute kidney injury network) criteria (**Table 1**) [6, 7]. However, timely detection of AKI with current RIFLE and AKIN criteria remains challenging because these definitions are entirely based on increases in Scr or decreases in urine output. Serum creatinine is insensitive for the early detection of AKI because the change in Scr does not discriminate the time and type of renal insult or the site and extent of glomerular or tubular injury [8].

The lack of reliable biomarkers for early detection of injury leads to delay in the introduction of treatment until well into the course of the renal injury. Therefore, many potential therapeutic agents showed little success in human studies [9-13]. There is no single or sequence of clinical interventions that will significantly improve renal function after onset of acute tubular injury or necrosis at the present time. Dialysis remains the only FDA-approved treatment option for established AKI.

1.3 Limitation of assessment of progressive loss of renal function

Chronic kidney disease (CKD) is characterized by a progressive loss of kidney function. The traditional methods of assessing kidney function decline are by serial measurement of Scr, reciprocal slope of Scr, or 24 hour creatinine clearance [14]. Changes in these markers appear relatively late in progressive CKD, at which point many interventions will be less effective than had they been introduced much earlier in the course of the injury. Even though proteinuria has

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been implicated as a good marker of progressive decline in kidney function [15], it may not present in many types of renal injury such as hypertensive renal disease and tubulointerstrial disease. Long-term renal outcome is determined by the severity of tubulointerstitial involvement. Sensitive tubular injury biomarkers may predict disease progression earlier and lead to more timely treatments before the development of elevated Scr or proteinuria. Thus, it is a logical step to evaluate whether sensitive AKI biomarkers can predict the progression of CKD in longitudinal studies.

1.4 New biomarkers under evaluation

Recently, several proteins emerged in animal and human studies as sensitive and specific AKI biomarkers capable of detecting injury early and grading its severity, including kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin 18 (IL-18), and L-fatty acid binding protein (L-FABP) [16-18]. The authors focus this review on KIM-1, which has shown promise in human studies not only in AKI, but also in CKD, kidney transplantation, and renal cell carcinomas (RCCs).

2] Kidney Injury Molecule-1

2.1 KIM-1 kidney expression and function in human

Human KIM-1 is a type 1 transmembrane glycoprotein containing a novel 6-cysteine immunoglobulin-like domain and a threonine/serine and proline-rich domain characteristic of mucin-like O-glycosylated proteins (**Figure 1**) [19]. KIM-1 is also known as hepatitis A virus cellular receptor 1 and T-cell immunoglobulin- and mucin-domain-containing molecule 1 [20-22]. The KIM-1 family consists of eight members in mice, six in rats and three in humans [23,

24]. KIM-1 protein is not detectable in normal kidney tissue or urine but is expressed at high levels in dedifferentiated proximal tubule epithelial cells in human and rodent kidneys after ischemic or toxic injury, and in RCC [19, 25-28]. The soluble KIM-1 protein that appears in the urine of humans is about 90kDa.

It is unclear whether expression of KIM-1 is related to the pathogenesis of the injury itself or marker of attempted recovery and repair. Ichimura *et al.* [29] demonstrated that KIM-1 is a scavenger receptor on renal epithelial cells, which play a central role in removal of apoptotic debris from tubular lumen. Furthermore, KIM-1 has been implicated in immune responses that regulate the development of autoimmune and allergic diseases [30-33]. KIM- 1 may play an important role in modulating the immunogenicity of RCC because RCC is an intrinsically immunogenic tumor [34]. In addition, KIM-1 has been associated in the development of interstitial fibrosis [35]. However, these associations are speculative and further studies are necessary to determine the functional role of KIM-1 in various renal diseases.

2.2 Methods of quantitation and stability of urinary KIM-1 proteins

Several methods have been used for urinary KIM-1 measurement in published studies. In the initial study, urinary KIM-1 protein was measured by direct sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibody and confirmed by western blot analysis [25]. Subsequent clinical studies have utilized customized direct sandwich ELISA or microsphere-based Luminex xMAP technology using a commercially available polyclonal KIM-1 antibody (R&D systems, Minneapolis, MN, USA) [36, 37]. Twenty to 30 µl urine samples are needed for both platforms. The inter- and intra-assay coefficients of variation are reported to be <10%. Recently, KIM-1 RenaSticks (Argutus Medical, Dublin, Ireland) were developed as a rapid diagnostic assay for rat and human kidney injuries [38]. However, there is very limited data for human urine samples. It appears to work well for high KIM-1 values.

Urinary KIM-1 protein did not degrade significantly up to 24 h in 4°C and in room temperature, and after prolonged storage at -80°C for 2 years with repeat freeze and thaw cycles [36]. Addition of protease inhibitor was not necessary to prevent degradation of urinary KIM-1 protein. This finding is critical to continuing validation studies of potential tandem AKI biomarkers.

3] Clinical applications of KIM-1

The following section discusses in detail the potential role of KIM-1 as a biomarker in various kidney diseases in humans.

3.1 Acute Kidney Injury

KIM-1 has been implicated as a urinary biomarker for acute tubular injury in both the hospital in-patient setting (**Table 2**) and before cardiac surgery or catheterization (**Table 3**).

3.1.1 Cross-sectional study. In a small cross-sectional study, Han *et al.* [25] demonstrated that the soluble form of cleaved KIM-1 could be detected in the urine of patients with established AKI, and found elevated urinary KIM-1 levels within 12 h after an initial ischemic insult, before the appearance of granular casts in the urine. Tissue expression of KIM-1 was also evaluated in biopsy samples of six patients with pathologically confirmed ATN and showed extensive expression of KIM-1 in the proximal tubules in all cases. Although promising, most of the urine samples were collected late into the course of kidney injury, some at the time of peak Scr. Thus, the relationship between severity of kidney injury and biomarker elevation could not be evaluated.

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Vaidya *et al.* [37] investigated the diagnostic performance of nine urinary biomarkers in a cross-sectional study. When compared to non-AKI patients (n=102), urinary KIM-1 showed strong diagnostic performance in detecting established AKI (n=102) with the area under the receiver operating characteristics curve (AUC-ROC) of 0.93. KIM-1 was also found to be a significant predictor of mortality ($P<0.001$), but not a predictor of RRT or the composite of mortality and RRT. Of note, no patients with underlying CKD were enrolled in the study, thus excluding a large population of subjects who frequently develop AKI in clinical practice. Urinary biomarkers were also measured at unknown and at variable times relative to AKI.

Overall, the above cross-sectional studies established that urinary KIM-1 could differentiate patients with established AKI from patients without AKI. However, a cross-sectional study gives no indication of whether urinary KIM-1 is useful for early detection and prognosis of AKI.

3.1.2 Prospective study. The following studies evaluated the temporal expression patterns of urinary KIM-1 from onset of renal insult. The diagnostic utility of urinary KIM-1 was evaluated for the early detection of postoperative AKI after pediatric cardiac surgery in a case control study [39] using samples from the same cohort in the NGAL study previously described [40]. In that study, urinary NGAL was measured and found to have an AUC-ROC of 0.99 and 1.00 at 2 and 4 h, respectively, following cardiopulmonary bypass (CPB). A subsequent study using all of the AKI cases and under half of the non-AKI cases showed urinary IL-18 to have an AUC-ROC of 0.61 at 4 h, 0.75 at 12 h, and 0.73 at 24 h following CPB [41]. Urinary KIM-1 had an AUC-ROC of 0.57 at 2 h, 0.83 at 12 h, and 0.78 at 24 h. Comparisons among the three biomarkers (NGAL from the original study and IL-18 and KIM-1 at later dates using frozen samples) should be made with caution for several reasons. The cohorts were all children with a small sample

size. CKD patients were excluded from the cohort. There were no patients who required dialysis or died.

Han *et al.* [36] also conducted a prospective study in 90 adults undergoing cardiac surgery at a single institution where AKI was defined as an increase in Scr of ≥0.3 mg/dl within 72 h after surgery. The AUCs to predict AKI (n=36) immediately and 3 h after operation were 0.68 and 0.65 for KIM-1; 0.61 and 0.63 for N-acetyl-β-glycosaminidase (NAG); and 0.59 and 0.65 for NGAL, respectively. Combining the three biomarkers enhanced the sensitivity of early detection of post-operative AKI compared with individual biomarkers. The AUCs for the three biomarkers combined were 0.75 and 0.78. However, direct comparison of Scr with urinary biomarkers for the detection of AKI was not done. In addition, how to combine multiple biomarkers for clinical use remains a challenge.

Liangos *et al.* [42] prospectively compared the performance characteristics of six urinary biomarkers in 103 patients following CPB for the early detection of AKI. KIM-1, NAG, NGAL, IL-18, cystatin C, and α-1 microglobulin were measured at 2 h postoperatively. AKI was defined as an increase in Scr by ≥50% in the first 72 h after termination of CPB. Among the biomarkers, KIM-1 had the highest predictive performance (AUC-ROC 0.78), and remained independently associated with AKI after adjusting for a preoperative AKI prediction score. This study was limited because there were a small number of patients and a single timed urine collection after operation. Only one patient who developed AKI required dialysis (n=13).

In a small prospective study, Malyszko *et al.* [43] evaluated the utility of urinary biomarkers for detection of contrast-induced nephropathy (CIN) in 140 patients without CKD after cardiac catheterization. Seventeen patients developed CIN, which was defined as an increase in SCr ≥ 25% from baseline within 48 h of cardiac catheterization. Urinary KIM-1 level was increased at 24 and 48 h post-catheterization for the CIN group, but without statistical

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significance. Serum and urinary NGAL levels increased significantly within the first 4 h of catheterization among patients with CIN. However, the study was very limited because there was no information on severity and outcome of CIN. In addition, there was no direct comparison of Scr with urinary biomarkers for the detection of CIN.

Biomarkers have been increasingly used in the critical care setting for diagnosis and risk stratification. High urinary KIM-1 expression was also associated with adverse clinical outcomes in patients with established AKI. In a cohort of 201 patients with established AKI, Liangos *et al.* [44] demonstrated that urinary KIM-1 and NAG were significantly associated with the clinical composite endpoints of death or RRT with an AUC-ROC of 0.78. However, when adjusted for disease severity (by APACHE II Score or MOF Score), or for comorbidities (including cirrhosis, sepsis, oliguria, and mechanical ventilation), urinary KIM-1 level lost its statistical significance.

Studies available at present are all insufficiently powered to establish a cutoff value that is predictive of AKI. All in all, larger prospective studies are necessary to validate the temporal expression pattern of urinary KIM-1 for early detection of AKI, and to establish how the temporal course relates to the onset, severity, and outcome of AKI.

3.2 Renal allograft

The early promise of KIM-1 as an AKI biomarker led to its study in other settings, including the kidney transplant population (**Table 4**). KIM-1 has been evaluated as a biomarker for delayed graft dysfunction, acute rejection and chronic damage through assessment of urinary excretion and kidney biopsy tissue expression.

KIM-1 expression has been implicated as a specific histological biomarker for diagnosing tubular injury on renal transplant biopsies. Zhang *et al.* [45] described tissue KIM-1 expression in 65 kidney allograft biopsies grouped by histological diagnosis (normal protocol biopsy, acute

tubular injury without cellular rejection, and acute cellular rejection). KIM-1 was expressed in 100% of cases with acute tubular injury without rejection and in 92% of cases of acute cellular rejection. There was, however, no urine KIM-1 data available for correlation with tissue KIM-1 expression. Meanwhile, Nogare et al. [46] also reviewed 59 kidney transplant biopsies, classified by Banff 1997 criteria into five groups: ATN; ATN with acute rejection episode (ATN/ARE); acute rejection episode without ATN (ARE); chronic calcineurin inhibitor toxicity (CNI); and interstitial fibrosis and tubular atrophy (IFTA). Tissue KIM-1 mRNA expression levels were increased in the CNI and IFTA groups but decreased in the other three groups. This result conflicts with earlier findings that KIM-1 is strongly associated with ATN.

Kidneys from brain death donors are susceptible to injury prior to kidney transplantation because brain death negatively affects hemodynamic stability and hormone regulation in the donor [47, 48]. Unlike previous studies in the allograft population, Nijboer et al. [49] evaluated KIM-1 before and after organ procurement. There was a 2.5-fold increase in allograft tissue KIM-1 gene expression among the donation after brain death (DBD) group (n=20) compared with living related group (n=20). Whereas there was no difference in urinary KIM-1 between the two groups before organ procurement, there was a twofold increase in urinary KIM-1 during organ recovery in the DBD group. Baseline urinary KIM-1 levels before procurement have been implicated as an independent predictor of Scr at 14 days and 1 year after transplantation. This study is limited by its small sample size and lack of clinical events noted during the procurement and transplant periods.

van Timmeren et al. [50] investigated the utility of urinary KIM-1 in predicting chronic kidney allograft loss in 145 kidney transplant recipients. Patients were divided into tertiles based on urinary KIM-1 levels, and higher tertile was associated with increased occurrence of graft loss, resulting in return to dialysis or re-transplantation. In terms of predictive performance for allograft loss within 5 years, AUC-ROC for KIM-1 level was 0.71, suboptimal when compared

with proteinuria (0.82) and creatinine clearance (0.89). There was no clinical information about etiologies of chronic kidney allograft dysfunction.

Hall *et al.* [51] evaluated the performance of urinary AKI biomarkers in predicting delayed graft function and renal recovery among deceased donor kidney transplants. This multi-center prospective study is the largest so far (n=91) to follow prospectively recipients immediately after transplant with serial urinary IL-18, NGAL, and KIM-1 levels. Urinary IL-18 and NGAL showed statistically significant difference between delayed graft function, slow graft function, and immediate graft function groups. Also, urinary IL-18 and NGAL were positive predictors for need for dialysis in 1 week and for graft recovery over 3 months after transplantations. Urinary KIM-1 performed poorly in this immediate post-transplantation cohort. Schroppel *et al.* [52] also investigated the post-transplant population by assessing both pre-perfusion tissue KIM-1 (n=115) and urinary KIM-1 (n=38) levels as predictors of delayed graft function. There was no correlation between urinary KIM-1 and tubular KIM-1 expressions in kidney allografts. Though pre-perfusion tissue KIM-1 expression inversely correlated with renal function at the time of organ procurement, there was no correlation between KIM-1 staining intensity and occurrence of delayed graft function.

As in AKI, the available studies assessing KIM-1 in the transplant population show that it is a sensitive tissue biomarker for injury. However, urinary KIM-1 did not perform better than traditional markers in predicting chronic allograft failure. Urinary KIM-1 also did not perform well in the immediate post-transplantation cohort as a biomarker for predicting delayed graft function and need for dialysis within the first week of kidney transplantation.

3.3 Renal cell carcinoma

As KIM-1 is strongly upregulated in proximal tubular cells in states of cell dedifferentiation, two studies evaluated the possible relationship of KIM-1 with RCC (**Table 5**). Han *et al.* [27]

investigated the association of tissue KIM-1 expression with RCC in 74 patients. Ninety-one per cent of clear cell RCC (n=56) stained positively for tissue KIM-1. Urinary KIM-1 levels were also significantly higher among RCC patients (n=42) compared with those with prostate cancer (n=10) or normal controls (n=30). Urinary KIM-1 levels decreased markedly or disappeared post-nephrectomy. The findings were limited by the retrospective nature of the study and small sample size.

A larger evaluation of tissue KIM-1 expression was published by Lin *et al.* [28] with 480 biopsies of various neoplasms analyzed. One hundred and seventy-nine biopsy samples of RCC were analyzed by tissue microarray, and 301 other samples (80 kidney and 221 non-kidney tumors) were evaluated by immunohistochemistry. Seventy-four per cent of clear cell, 93% of papillary, and 78% metastatic RCC were differentiated from chromophobe RCC and oncocytomas by markedly different degrees of KIM-1 expression. Of note, tissue KIM-1 was found to be highly positive in certain non-renal cancers, including 93.8% of clear cell carcinoma of the ovary and 33% of clear cell carcinoma of the uterus. Though this retrospective analysis provided new possible utilities of KIM-1 in detecting non-renal cancers, further studies are needed to clarify the mechanisms and clinical importance of this finding.

Study in a larger number of patients is necessary to establish the sensitivity and specificity of KIM-1 in RCC, as well as the temporal relationship between diagnosis of RCC and rise in urinary KIM-1 levels. If further studies confirm association of KIM-1 with subtypes of RCCs, this may provide a non-invasive means of early detection and a potential screening tool in high-risk patients.

3.4 Chronic kidney disease

KIM-1 has also been evaluated as a predictor of disease progression in CKD (**Table 5**). van Timmeren *et al.* [35] studied the KIM-1 expression in human kidney biopsies (n=102) and its

correlation with urinary KIM-1 (n=53) at the time of biopsy in patients with various types of CKD. Tissue and urinary KIM-1 expression correlated positively with interstitial damage, inflammation, and Scr, but did not correlate with proteinuria. To explain this, the authors suggest that not all proteinuria is accompanied by tubulointerstitial damage and progressive decline in renal function, using minimal change disease (MCD) as an example. Waanders *et al.* [53] also studied proteinuria and KIM-1 by analyzing effect of anti-proteinuric regimens of diet and RAAS inhibition on KIM-1 and NAG. In non-diabetic Stage III CKD, decreased urinary KIM-1 correlated with decreased proteinuria in each interventional group studied, but not with blood pressure or creatinine clearance. The study suggests that improvement of proteinuria will result in decreased tubulointerstitial damage as reflected by urinary KIM-1. Major limitations of this study include its small sample size, exclusion of diabetics, short duration (maximum six weeks for any given intervention), and single urinary sample obtained after intervention. Prospective studies that extend beyond six weeks are necessary in assessment of CKD and response to therapy.

Conflicting results exist regarding the role of KIM-1 in evaluation of CKD, particularly its relationship with proteinuria. The question remains whether urinary KIM-1, used in tandem with urine protein-to-creatinine ratio, can enhance detection of kidney disease progression and future need for dialysis, and whether such information can lead to more aggressive treatment and improved outcome.

4] Conclusion

KIM-1 is expressed exclusively in injured proximal tubules and shed in the urine during tubular injury, making it readily detectable in the urine. The variety of human studies evaluating tissue and urinary KIM-1 across various kidney diseases has provided insight into its utility. KIM-1

expression is not only highly elevated in acute tubular injury, but also detectable in various etiologies of AKI, CKD, the kidney transplant population, and RCC. New questions arose from these studies, namely the mechanism of KIM-1 expression in non-renal disorders, from hospitalization without history or biochemical marker of kidney disease to non-renal clear cell carcinomas.

Available studies have also highlighted several limitations of KIM-1 in the detection and prediction of kidney injury and disease, which preclude the mainstream acceptance of KIM-1 in the clinical setting. Most of the human studies come from single centers and from homogeneous patient populations. Urinary KIM-1 has been tested only in small studies and limited clinical situations. Most studies assessing AKI exclude CKD patients. Existing studies have been insufficiently powered to establish a cutoff value that is predictive of AKI. In addition, there is at present very limited data available regarding temporal expression patterns of urinary KIM-1 in the various clinical settings of AKI from onset of renal insult or utility for differentiating causes of AKI. Furthermore, there is no report on detecting soluble KIM-1 in serum samples of AKI patients. Studies investigating CKD are few, and the results are conflicting with regards to association of KIM-1 with proteinuria. Tissue KIM-1 is a sensitive biomarker for kidney injury in the kidney transplant population, but existing studies have not shown ability to discriminate between types of injury or to predict graft failure. So far, clinical utility of KIM-1 in human studies has been more promising in AKI and RCC populations than in kidney transplant or CKD populations.

5] Expert Opinion

The particular area of biomarker research that remains most interesting is in AKI population because they can have their greatest impact on clinical care. The goal of early detection of AKI

is the introduction of therapy early enough in the disease process to reduce the high mortality rate associated with AKI.

The search for new AKI biomarkers has been evolving rapidly with advancement in modern technologies. Recently, several protein biomarkers, including KIM-1, NGAL, IL-18, cystatin C, and L-FABP, emerged through the application of functional genomics and proteomics to human and animal models of AKI. However, several requirements must be met for their use in daily clinical practice. The AKI biomarkers must: i) allow for early detection of kidney injury; ii) identify severity of AKI; iii) provide a rationale for risk stratification for clinical studies including the identification of patients at risk for AKI; iv) guide timing of therapy; v) reflect improvement and worsening of the kidney injury; and vi) be amenable to quick and reliable measurement at the bedside or in the clinical laboratory. Unfortunately, none of these biomarkers has demonstrated a clear benefit in various types of AKI. The published clinical studies of urine KIM-1 are small so far, and there is no clear evidence whether urinary KIM-1 is an effective AKI diagnostic test in humans.

The heterogeneity of AKI and existing published data suggest that more than one biomarker may be necessary to obtain sufficient sensitivity and specificity for AKI screening. There is emerging evidence that combining multiple biomarkers may allow early detection of AKI [36, 39], but how to combine multiple biomarkers for clinical use remains a challenge. As such, there is at present no standard way to combine the multiple biomarkers for clinical use. The ultimate goal of a more sensitive biomarker or panel of biomarkers is improved early detection and monitoring of AKI and other kidney disease, which will lead to improved outcomes through earlier therapeutic intervention and the re-evaluation of other pharmacologic agents that have shown promise in experimental models.

KIM-1 or other biomarker research is past cross-sectional measurement unrelated to a clinically or biochemically defined end point. The inherent benefit of AKI biomarkers is for

making an early diagnosis of AKI that correlates with future declines in function as detected by changes in Scr, not when Scr is already grossly elevated. Future studies must continue to move from assessment of KIM-1 to more directed measurement in close temporal relationship to injury. Larger prospective multi-center studies are necessary to validate the temporal expression pattern of urinary KIM-1 for early detection of AKI, and to elucidate how this temporal course relates to the onset, severity, and outcome of AKI. Recently, the NIH launched the Translational Research Investigating Biomarkers Endpoints in AKI study, which is a multi-center prospective observational study to validate biomarkers for early diagnosis and prognosis of AKI after cardiac surgery among adult and pediatric patients. More such prospective studies that are sufficiently powered to establish a cutoff value that is predictive of AKI, especially with severe injury, are needed. Then a new standard definition of AKI not based on a change in Scr can be proposed. Multiple challenges have prevented the clinical use of new biomarkers in early detection of AKI. The use of an imperfect marker in the authors' definition of AKI, Scr, has certainly contributed to this difficulty. A new definition of AKI should be based on multiple biomarkers, used in the setting of careful clinical assessment and other clinical and laboratory parameters, to detect initial renal injury within minutes to hours.

Once a more sensitive biomarker or panel of biomarkers for AKI is validated for clinical use, the next challenging tasks are the development of a rapid assay and the selection of a suitable patient group for pharmacologic treatment. Current quantification methods include ELISA, Luminex, and nephelometry. Rapid assays must improve upon current technologies and provide a quick and reliable measurement at the bedside or clinical laboratory for the detection of AKI. Then multiple therapeutic possibilities that showed promise in animal studies, but failed in human studies, can be revisited in clinical trials. Furthermore, it is critical to know the timing of the kidney insult because previous animal studies and failed attempts of human intervention clinical trials demonstrate that introduction of treatment should precede the rise of Scr and be

very early after the insult. This may limit the utility of AKI biomarkers and potential pharmacologic treatments to patients with hospital acquired AKI, including post-operative or contrast-dye induced AKI.

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Disclosures

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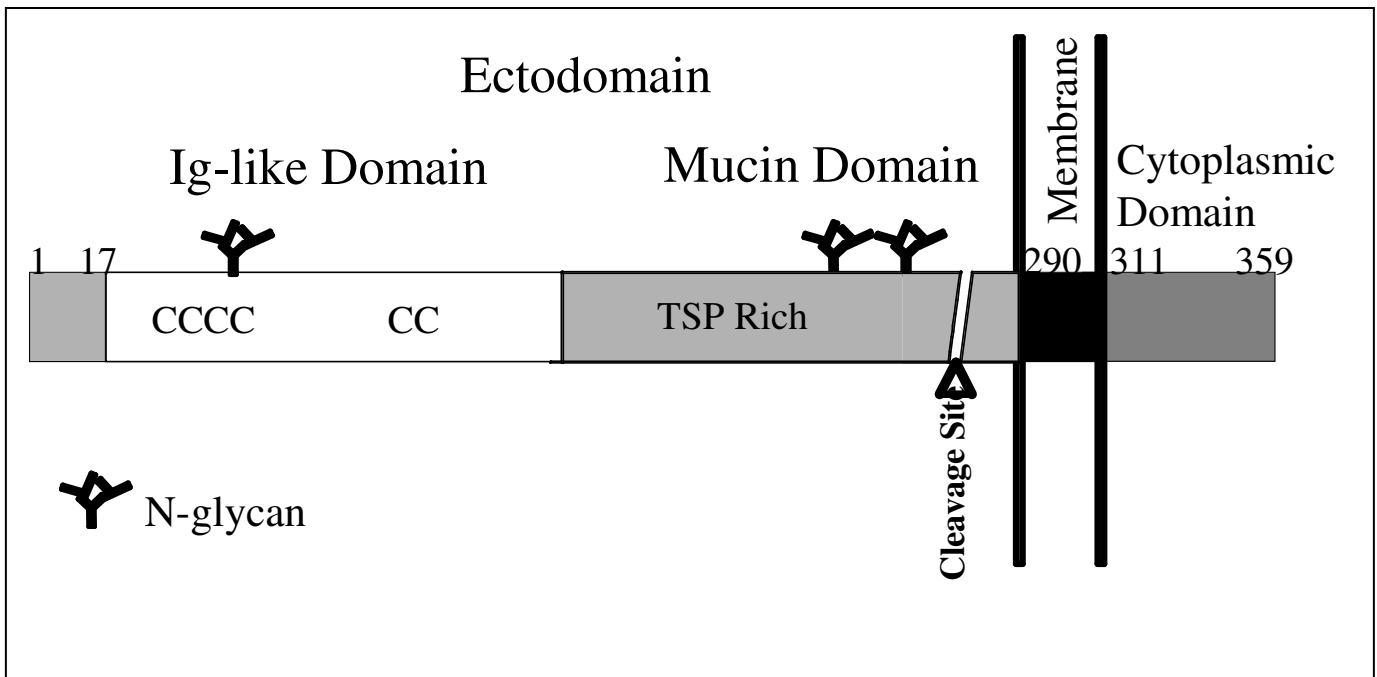
Figure

Figure 1. **Structure of human KIM-1**. The protein is a type 1 transmembrane glycoprotein with most of the protein made of an extracellular domain that consists of a signal peptide, immunoglobulin-like domain and a mucin domain. The protein is cleaved by a metalloproteinase and the ectodomain (90 kDa) appears in the urine of humans with acute kidney injury and renal cell carcinoma.

Table 1. RIFLE and AKIN Staging Criteria for AKI

Stage	Creatinine Criteria	Urine Output Criteria
1	Increase in Scr of ≥ 0.3 mg/dl or increase in Scr of 1.5 to 2.0 fold from baseline	<0.5 ml/kg per h for 6 h
2	Increase in Scr of 2.0 to 3.0 fold from baseline	<0.5 ml/kg per h for 12 h
3	Increase Scr to > 3.0 fold from baseline or Scr > 4 mg/dl with an acute rise of ≥ 0.5 mg/dl	<0.3 ml/kg per h x 24 h or anuria for 12 h

Stages removed from RIFLE criteria in AKIN stages

Loss	Persistent acute renal failure=complete loss of kidney function > 4 weeks
ESRD	End-stage kidney disease (> 3 months)

AKIN, acute kidney injury network; AKI, acute kidney injury; RIFLE, risk, injury, failure, loss, ESRD; Scr, serum creatinine

Table 2. Clinical study for KIM-1 as a Biomarker for Acute Kidney Injury in Hospitalized Patients

Clinical Study	Year	Study population	Type of study	Comparison biomarkers	Measurement	Method of detection	Major findings	Limitations
...n *et al.* [25]	2002	Hospitalized patients with AKI *vs* non-hospitalized controls (n=40)	Cross-sectional	alkaline phosphatase, γ-glutamyl-transferase, total protein	Tissue KIM-1 & Urine KIM-1	Sandwich ELISA	High KIM-1 expression in acute tubular injury. KIM-1 is elevated in AKI compared to non-AKI group	Small sample size. Most urine samples were drawn late into course of kidney injury.
...angos *et al.* [..4]	2007	Hospitalized patients with AKI (n=201)	Prospective	NAG	Urine KIM-1	Luminex	KIM-1 and NAG associated with the clinical composite of death and need for RRT.	Timing of measurement dependent on time of renal consult
...idya *et al.* [..7]	2008	Hospitalized patients with AKI *vs* non-hospitalized controls (n=204)	Cross-sectional	NGAL, IL-18, NAG, HGF, cystatin C, VEGF, IP-10, total protein	Urine KIM-1	Sandwich ELISA	KIM-1 has a good discriminatory characteristic of AKI biomarker. Not a predictor of RRT or the composite of mortality and RRT.	Small number of hospitalized patients as a control group. Elevated KIM-1 levels in hospitalized patients without AKI. No inclusion of CKD patients.

AKI, acute kidney injury; CKD, chronic kidney disease; ELISA, enzyme-linked immunoabsorbent assay; HGF, hepatocyte growth factor; IL-18, interleukin 18; IP-10, interferon-inducible protein-10; KIM-1, kidney injury molecule-1; NAG, N-acetyl-β-glucosaminidase; NGAL, neutrophil gelatinase associated lipocalin; RRT, renal replacement therapy; VEGF, vascular endothelial growth factor.

Table 3. Clinical Study for KIM-1 as a Biomarker for Post-operative Acute Kidney Injury and Contrast-Induced Nephropath

Clinical study	Year	Study population	Type of study	Comparison biomarkers	Measurement	Method of detection	Major findings
Han *et al.* [39]	2008	AKI *vs* non-AKI (n=74). Children with cardiac surgery (n=40)	Cross-sectional and Case-control	NAG, MMP-9	Urine KIM-1	Sandwich ELISA	KIM-1 can allow early diagnosis of post-operative AKI before a rise in Scr
Liangos *et al.* [42]	2009	Adults with cardiac surgery (n=103)	Prospective	NAG, NGAL, Cystatin C, IL-18, Alpha-1 microglobulin	Urine KIM-1	Sandwich ELISA	KIM-1has the best predictive value for detection of AKI.
Han *et al.* [36]	2009	Adults with cardiac surgery (n=90)	Prospective	NAG, NGAL	Urine KIM-1	Sandwich ELISA	A panel of AKI biomarkers may improve the early detection of postoperative AKI
Malyszko *et al.* [43]	2009	Adults with cardiac catheterization (n=140)	Prospective	NGAL, IL-18, Cystatin C, L-FABP	Urine KIM-1	Commercial kit	KIM-1 tends to be higher a 24 and 48 hours post-catheterization in CIN group, but it is not statistically significant

AKI, acute kidney injury; CIN, contrast-induced nephropathy; CKD, chronic kidney disease; ELISA, enzyme-linked immunoabsorbent assay; IL-18, interleukin 18; KIM-1, kidney injury molecule-1; L-FABP, L-fatty acid binding protein; MMP-9, matrix metalloproteinase 9; NAG, N-acetyl-β-glucosaminidase; NGAL, neutrophil gelatinase associated lipocalin; Scr, serum creatinine.

1 as a biomarker in transplant population

Type of study	Other Biomarkers	Measurement	Detection Method	Major findings	Limitations
Prospective	No	Urine KIM-1 (24-hour)	Luminex	Highest tertile of KIM-1 independent predictor of graft loss when adjusted for age, creatinine clearance and proteinuria	Single center. Single 24-hour urine sample. Wide variation of time of inclusion allows for healthy survivor bias. No histological correlation.
Retrospective	No	Tissue KIM-1	IHC	KIM-1 expressed in ATI and ACR; KIM-1 in 28% of normal biopsy; KIM-1 correlated with renal dysfunction	Among normal protocol biopsies, no clinically significant difference in renal function regardless of KIM-1 staining.
Prospective	No	Tissue KIM-1 mRNA & Urine KIM-1	Real-time PCR Sandwich ELISA	Significant increase KIM-1 in DBD kidneys and urine after procurement.	Single center study. Small sample size. Precise timing of when samples taken not specified.
Retrospective	No	Tissue KIM-1 mRNA	Real-time PCR	KIM-1 mRNA levels are increased in the CNI and IFTA categories, but not in ATN or	Non-diverse population. Single center. Indication or timing of biopsy from injury

				ARE.	not specified. No clinical correlation.
Prospective	NGAL, IL-18	Urine KIM-1	Sandwich ELISA	KIM-1 is not correlated to graft function. IL-18 and NGAL statistically significant difference between three groups.	Small sample size.
Prospective	No	Tissue KIM-1 mRNA and protein & Urine KIM-1	IHC PCR Luminex	No significant correlation between KIM-1 and presence or absence of DGF. KIM-1 is not correlated to graft function.	Small sample size.

ACR, acute cellular rejection, ARE, acute rejection episode; ATI, acute tubular injury; ATN, acute tubular necrosis; CNI, calcineurin inhibitor toxicity; CRT, cadaveric renal transplant; DBD, donation after brain death; DGF, delayed graft function; IFTA, interstitial fibrosis and tubular atrophy; IGF, immediate graft function; IHC, immunohistochemistry; LRT, living related transplant; PCR, polymerase chain reaction; SGF, slow graft function

Table 5. Clinical Study of KIM-1 as a Biomarker in RCC and CKD

Clinical Study	Year	Study Population	Type of Study	Other Biomarkers	Measurement	Detection Method	Major Findings
Han *et al.* [27]	2005	Renal tumor (n=82) *vs.* Non-renal tumor (n=484)	Retrospective & Prospective	RCC-Ma	Tissue and Urine KIM-1	IHC, ELISA	Tissue KIM-1 in clear cel RCC vs non-RCC. Urine KIM-1 in RCC. Marked decrease/disappearance K in RCC post-nephrectomy
Lin *et al.* [28]	2007	Renal tumor (n=259) vs. Non-renal tumor (n=221)	Retrospective	No	Tissue KIM-1	IHC	Increased KIM-1 in clear papillary cell, and metast RCC. KIM-1 expression clear cell type non-renal cancers.
van Timmeren *et al.* [35]	2007	CKD tissue (n=102) and urine (n=57)	Retrospective	No	Tissue and Urine KIM-1	IHC, ELISA	Urine KIM-1 correlated w tissue KIM-1, negative correlation with renal function; no correlation w proteinuria.
Waanders *et al.* [53]	2009	Stable CKD Stage III, proteinuric, non-diabetic (n=34)	Prospective post-hoc analysis	NAG, Total protein	Urine KIM-1 (24 hour)	Luminex	Decrease in KIM-1 in all treatment groups. Correla with decreased proteinuri not renal function.

CKD, chronic kidney disease; ELISA, enzyme-linked immunoabsorbent assay; KIM-1, kidney injury molecule-1; NAG, N-acetyl-β-glucosaminidase; RCC, renal cell carcinoma; RCC-ma, RCC marker (monoclonal antibody to a proximal tubule renal antigen)