

Biomarkers in Acute Kidney Injury

Shilpushp J Bhosale¹, Atul P Kulkarni²

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BACKGROUND

Despite significant progress in the management of acute kidney injury (AKI), it still carries high morbidity and mortality. The major reason for this is the difficulty in identifying renal injury and delay in diagnosis. The mortality rates in AKI patients ranges from 10% in hospitalized patients to 60% in patients admitted to the intensive care unit (ICU) depending on underlying conditions. Diagnosis of AKI poses significant challenges and difficulties, because unfortunately it is still diagnosed on the basis of serum creatinine and urine output (UO) which are functional markers rather than markers of renal injury.¹

Significant changes have occurred in defining AKI, with the advent of various criteria for classifying AKI, risk, injury, failure, loss of kidney function, and end-stage kidney disease (RIFLE), and acute kidney injury network (AKIN), and now the kidney disease improving global outcomes (KDIGO) criteria. Our understanding has thus improved that AKI is continuum of disease and also even subclinical or mild reductions in kidney function can lead to poor patient outcomes. The KDIGO criteria may increase the sensitivity of diagnosis by using small change in creatinine; however, there are significant limitations to using serum creatinine and UO to diagnose AKI. We know that serum creatinine rises very late after renal injury and is not proportional to the extent of renal injury until a steady state has been reached. Creatinine rise is again influenced by various factors such as renal reserve, extent of tubular damage, intravascular volume status, patients muscle mass and nutrition, hemodynamic changes, and fluid shifts.² Creatinine rise does not help in quantification of injury and degree of renal dysfunction or differentiate renal azotemia from AKI and stable chronic kidney disease. It is also poor in predicting renal recovery and does not help in making decisions about treatments, such as need for renal replacement therapy. Urine output may be a better time-sensitive marker of glomerular filtration rate (GFR), as it often decreases before serum creatinine concentration increases. However, it has to be noted that severe AKI can exist in spite of UO being normal. It is commonly seen that in critically ill AKI patients diagnosed by UO invariably have fluid overload.

BIOMARKERS OF AKI

Traditionally we have used biochemical markers and microscopic examination of the urine to diagnose the cause of AKI. We checked the granular or epithelial casts in acute tubular necrosis and fractional excretion of sodium or urea to differentiate prerenal from renal causes of AKI such as renal tubular injury. However, these tests are not very sensitive nor specific.

The KDIGO guidelines suggest the use of newer biomarkers to identify AKI at its earliest stage before the loss of organ function,

^{1,2}Division of Critical Care Medicine, Department of Anaesthesiology, Critical Care and Pain, Tata Memorial Hospital, Homi Bhabha National Institute, Parel, Mumbai, India

Corresponding Author: Shilpushp J Bhosale, Division of Critical Care Medicine, Department of Anaesthesiology, Critical Care and Pain, Tata Memorial Hospital, Homi Bhabha National Institute, Parel, Mumbai, India, Phone: +91 9619310657, e-mail: shilbhosale@gmail.com

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so that the care bundle can help prevent AKI, especially before the associated complications become irreversible.³ Kellum et al. proposed calling AKI as “kidney attack” to instill a sense of urgency in healthcare team, considering that AKI sometimes has worse outcomes than acute myocardial infarction (AMI).⁴ Similar to AKI, AMI at present remains a clinical diagnosis supported by biomarkers. In the same year, the KDIGO clinical practice guideline for AKI was published.⁵ Six years later, Kellum et al. noted the improvement in outcomes of patients with AKI, when strategies such as KDIGO bundle, early detection of renal stress with biomarkers, and remote ischemic preconditioning were implemented.⁶ Most functional, damage or preinjury phase biomarkers are either low-molecular-weight proteins that undergo glomerular filtration or enzymes released by tubular cells (or inflammatory mediators).⁷

The development of newer stress biomarkers such as cell cycle arrest markers that measure cellular stress even before damage or loss of function (preinjury phase) is quite promising.⁶ Various clinical studies using single or even combined biomarkers have been conducted so far to validate these markers, though the majority of these studies are based on cardiac surgery patients and in children where the incidence of AKI is very high. We have to be clear at the outset that multiple hurdles are involved in using the biomarkers of AKI in the clinical setting, i.e., the complexity and diversity of the pathogenesis, the progress, and the way the biomarkers are used. We may get a definitive biomarker in the future, but at present we are far away from reaching that stage.

A list of biomarkers for detecting renal injury is given in Table 1.^{8–10}

Neutrophil Gelatinase-associated Lipocalin (NGAL)

The NGAL is a 25-kDa glycoprotein that is bound to neutrophil gelatinase. It is normally present in tissues of lungs, stomach, colon, and proximal tubular epithelial cells. In early stages, NGAL is expressed in response to ischemic or nephrotoxic kidney injury and may even serve as a prognostic marker for AKI.^{11,12} Studies show

Table 1: List of biomarkers for detecting renal injury

Proximal tubule	Glomerulus	Preinjury biomarkers
<ul style="list-style-type: none"> Albumin IL-18 GST-α (urinary) Clusterin Cystatin C (urinary) KIM-1 α-1 Microglobulin β-2 Microglobulin NGAL HGF Netrin-1 Osteopontin NHE-3 Cyr61 L-FABP Exosomal fetuin-A NAG RBP NHE-3 	<ul style="list-style-type: none"> Albumin Total protein α-1 Microglobulin β-2 Microglobulin Cystatin C (urinary) <p>Loop of Henle</p> <ul style="list-style-type: none"> Osteopontin NHE-3 <p>Distal tubules</p> <ul style="list-style-type: none"> GST-μ/π NGAL Osteopontin Clusterin H-FABP Calbindin D-28 <p>Collecting duct</p> <ul style="list-style-type: none"> Calbindin D-28 	<ul style="list-style-type: none"> IGFBP-7 @ TIMP-2 @ DKK1-4 (DKK-3)* (serum, urinary) Hemojuvelin (HJV) (urinary) Micro-RNAs (U) (urinary) Wnt (serum, urinary) <p>Others</p> <ul style="list-style-type: none"> Cytochrome-C (urinary) Epidermal growth factor (urinary) Malondialdehyde (urinary)

DKK-3, Dickkopf-3, *DKK-3 is used most commonly, NHE-3, Na⁺/H⁺ exchanger isoform 3; NAG, N-acetyl-beta-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; RBP, retinol-binding protein; Cyr 61, cysteine-rich 61; IL-18, interleukin 18; GST-α, glutathione S-transferase-α; HGF, hepatocyte growth factor; L-FABP, L-type fatty acid-binding protein; IGFBP-7, insulin-like growth factor-binding protein-7; TIMP-2, tissue inhibitor of metalloproteinase 2; @ [IGFBP-7] × [TIMP-2] are always used together and are marketed as such; H-FABP, heart fatty acid-binding protein

that NGAL rises within 3 hours after injury and reaches the peak level at 6 hours. The levels may remain elevated for up to 5 days. The main drawback is that NGAL is also secreted from other tissues in stress and hence is not very specific for kidney injury. Mishra et al. evaluated the urinary NGAL in patients undergoing corrective cardiac surgeries and found that urinary NGAL is highly sensitive and can predict AKI in as early as 2 hours after surgery with specificity was 0.98 with a cutoff value of 50 µg/L.¹³

Cystatin C

Cystatin C is a cysteine protease inhibitor that is synthesized by nucleated cells. It is filtered by the glomerular cells and gets completely reabsorbed in the proximal tubule, which makes it a better marker than creatinine for GFR estimation, especially in children. Cystatin C levels increase much before the creatinine level in the patients with AKI.¹⁴ The drawback is that levels are influenced by age, sex, steroids, thyroid disease, smoking and alcohol use, inflammation, and malignancy, thus limiting its widespread use.¹⁵

Nickolas et al. studied the urinary biomarkers NGAL, Kidney injury molecule-1 (KIM-1), L-type fatty acid-binding protein (L-FABP), interleukin 18 (IL-18), and cystatin C in patients who presented to the emergency department. They found that NGAL had 81% specificity at cutoff of 104 ng/mL for diagnosis of AKI, and also NGAL and cystatin C were able to distinguish persistent AKI from transient AKI.¹⁶ In another study of 72 adults undergoing elective cardiac surgery, Koyner et al. found that urinary NGAL and cystatin C levels increased 6 hours after ICU admission, suggesting early AKI, and that the urinary levels were better predictors than serum levels.¹⁷

Liver-type Fatty Acid-binding Protein

Liver-type fatty acid-binding protein is a 14-kDa protein that is synthesized by the liver, intestine, and proximal renal tubule epithelium. It is a marker of hypoxic injury to the renal cells. It may be protective and has antioxidant properties, and it therefore may get expressed in response to hypoxia. It binds to products of lipid oxidation and protects against tubular injury and oxidative damage. Susantitaphong et al. confirmed that urinary L-FABP can be used for diagnosis of AKI with a sensitivity of 74.5% and specificity of 77.6%. Its sensitivity for predicting the need for dialysis was 69.1%.¹⁸

Kidney Injury Molecule-1

Kidney injury molecule-1 (KIM1) is a 38.7-kDa transmembrane tubular glycoprotein which has an extracellular immunoglobulin domains and intracellular signaling protein for tyrosine phosphorylation. Kidney injury molecule-1 is expressed in response to ischemic proximal tubular injury and the extracellular domain gets secreted into the urine. Urinary KIM-1 has shown to be very sensitive and specific marker of proximal tubular kidney injury and can also distinguish ischemic acute tubular necrosis from prerenal azotemia.¹⁹ Han et al. looked at the expression of KIM-1 in six patients who had biopsy-proven acute tubular necrosis, and it was found that urinary KIM-1 levels were significantly higher in patients who had ischemic acute tubular necrosis.²⁰

Interleukin 18

Interleukin 18 is a 22-kDa proinflammatory cytokine that is also expressed in distal collecting tubular cells in response to ischemia. It can differentiate tubular damage and acute tubular necrosis

from prerenal azotemia and chronic kidney disease.²¹ Studies have shown that IL-18 is a sensitive, early biomarker of AKI, especially post cardiopulmonary bypass with its levels rising well before creatinine. The area under receiver–operating characteristic (ROC) curve is 73%, with the IL-18 values >100 pg/mL. It is a strong predictor of AKI in the subsequent 48 hours.²² Its drawback is that, since it is an inflammatory marker, it increased in many other conditions such as sepsis, endotoxemia, inflammatory, and autoimmune diseases.

Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) and Insulin-like Growth Factor-Binding Protein-7 (IGFBP-7)

Both TIMP-2 and IGFBP-7 are two newer cell cycle arrest biomarkers (Nephrocheck) referred to as renal troponin. They are used in combination and have been recently approved by the Food and Drug Administration.²³ In studies it has been found that cell cycle arrest helps in adequate repair by preventing division of cells with damaged DNA. This is a protective mechanism to avoid exposure to stress and injury. The TIMPs are known to inhibit matrix metalloproteinases and endothelial cell proliferation whereas matrix metalloproteinases are known to cause fibrosis, matrix weakening, angiogenesis, and apoptosis. It also facilitates kidney recovery after ischemic insult.

Both TIMP2 and IGFBP7 are G1 cell cycle arrest proteins that are synthesized in response to ischemic or toxic injury cell. They are secreted in injured renal tubular cells, blocking endothelial cell proliferation by activation of kinases. They also affect the availability of insulin growth factors, which are required for tumor suppression and cell senescence or aging process during the repair phase of AKI. However, if cell cycle arrest is prolonged, then it leads to fibrosis and chronic kidney disease.

The TIMP-2 is a 21-kDa protein that has antiapoptotic and proliferative properties. While IGFBP7, a 29-kD protein, is IGF-1 receptor antagonist that causes tumor suppression and regulation of cellular aging by inhibiting signaling of kinases. Thus, these cell cycle biomarkers are stress markers that detect ischemic or toxic cell injury causing AKI early within 24 hours.

The TIMP2/IGFBP7 test with a cutoff of > 0.3 is a strong predictor of AKI and value > 2.0 indicates higher kidney stress and probable AKI within 12–24 hours.²⁴ The Sapphire study in 728 critically ill patients with sepsis, shock, major surgery, and trauma, Kashani et al. validated TIMP-2 and IGFBP7 with highest combined ROC of 0.80 (0.76 and 0.79, respectively). They also found that TIMP-2 and IGFBP7 were superior to other markers of AKI such as NGAL and KIM-1 ($p < 0.002$).²⁵

The discussion on biomarkers will remain incomplete without alluding to the Translational Research Investigating Biomarker Endpoints–ICU (TRIBE-ICU) trial. The translational research investigating biomarker endpoints in acute kidney injury (TRIBE-AKI) trial assessed long-term kidney-related outcomes after elective cardiac surgery. They included 1,199 adults and evaluated the performance of urinary IL-18, urinary albumin to creatinine ratio (ACR), and urinary and plasma NGAL on the day the patients developed AKI as diagnosed by AKIN criteria. They found that three of these biomarkers, i.e., urinary IL-18, urinary ACR, and urinary and plasma NGAL were able to predict the progression of AKI independently. Thus, patients who have a quantitative increase in these biomarkers had higher risk of progression of AKI with worsening at AKIN stage.²⁶

CONCLUSION

Numerous biomarkers have been used for early detection of AKI but failed to elicit troponin-like performance. It is also seen that unfortunately none of the biomarkers have been truly specific for AKI. The relationship between increasing damage and decreasing function may not be as linear as initially thought to be. Most discouraging aspect is that in practice in spite of early detection of AKI with biomarkers, it does not improve the outcomes of these patients as the therapeutic options that we have are limited to renal replacement therapy. Early detection of AKI with biomarkers can improve the prognosis only by better risk stratification and the use of appropriate (so far unavailable) therapy.

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