

# Review Article

## Biomarkers of Acute Kidney Injury

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Chettinad Health City Medical Journal 2018; 7(2): 40 - 43

### Abstract

Acute kidney injury (AKI) is triggered by number of factors like ischaemia, nephrotoxins etc and is associated with increased morbidity and mortality. AKI diagnosis is usually based on the rise in serum creatinine or reduction in urine output. Though creatinine is used as an indicator of kidney function, it is a suboptimal marker of injury as it rises only after around 50% of function loss. The delay in diagnosis of kidney injury prevent timely management decisions raising the demand for sensitive, specific and reliable biomarkers that can stratify correctly the extend of renal damage. A number of novel biomarkers are currently available to represent kidney injury and kidney function. Here we make an effort to present the main features of emerging biomarkers. An analytical approach attempted to know whether the new biomarkers diagnose acute kidney injury earlier than traditional tests, whether they can identify patients who need renal replacement therapy, does it improve patient outcome and when it should be measured.

**Key Words:** Acute kidney injury, Biomarkers, Renal replacement therapy

### Introduction

Acute kidney injury (AKI) previously termed as acute renal failure represents an acute decline in renal function and is associated with a number of short term and long term complications.<sup>1-3</sup> Diagnosing AKI in ICU setting is a great challenge. Due to late diagnosis, AKI leads to increased mortality, prolonged hospital stay and cost.<sup>2,4</sup> Series of changes occur at molecular and cellular levels that leads to renal dysfunction.<sup>4</sup> Serum creatinine levels and serum creatinine based formulas are used to assess kidney function until now. Serum creatinine concentration varies according to age, sex, muscle mass, medications and hydration status and concentration and does not change until significant loss of kidney function.<sup>5</sup> Hence creatinine based decision making in critically ill patients is debatable.<sup>4</sup> There is need for early recognition of renal dysfunction with sensitive and specific biomarkers which would be useful for the early diagnosis of AKI, prediction of clinical outcomes and response to treatment.<sup>3,4</sup> Early diagnosis may prevent progression to CKD by limiting level of injury and facilitating early recovery.<sup>6</sup> Studies over past decade shows that novel biomarkers are better than serum creatinine measurement. Currently many prospective studies are being done, to relate biomarkers with short term outcomes such as in-hospital mortality, need for renal replacement therapy and length of stay. Apart from that, long term outcomes such as cardiovascular events and CKD are also predictable.<sup>3</sup>

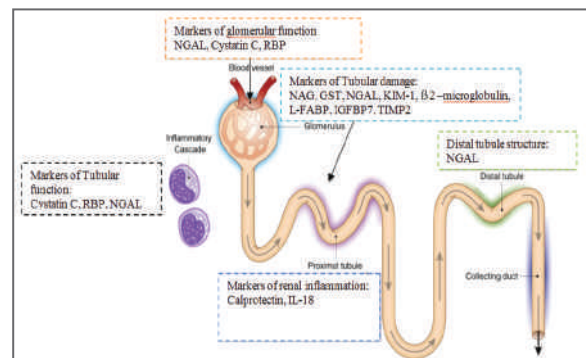


Fig 1: Physiology of biomarkers of AKI. Adapted from.<sup>6</sup> AKI, acute kidney injury, L-FABP, liver fatty acid binding protein, IGFBP-7, insulin-like growth factor binding protein 7; IL-18, interleukin-18; RBP - Retinol Binding Protein; NAG, N-acetyl-β-D-glucosaminidase; KIM-1, kidney injury molecule-1; TIMP-2, tissue inhibitor metalloproteinase-2, NGAL, neutrophil gelatinase-associated lipocalin.

### Pathophysiology of AKI and biomarkers

Based on etiology, AKI can be classified as prerenal, renal and postrenal.<sup>7</sup> The injury may be due to various insults such as decreased perfusion, major surgery, nephrotoxins, tubulointerstitial inflammation and edema. The most common nephrotoxic agents are aminoglycosides followed by analgesics, contrast media, chemotherapeutic agents and environmental contaminants.

The process of kidney injury goes through various reversible stages starting from risk to damage and finally progresses to kidney failure. In sepsis, ischemia and toxins contribute to the injury in majority of case. Ischemic or toxic insult leads to altered autoregulation causing renal vasoconstriction which in turn leads to decreased renal perfusion.<sup>1</sup> Vascular changes may be due to increased cytosolic calcium in the afferent arterioles of the glomerulus. This observation is substantiated by the reversal of loss of auto regulation by intrarenal calcium channel blockers.<sup>8</sup>

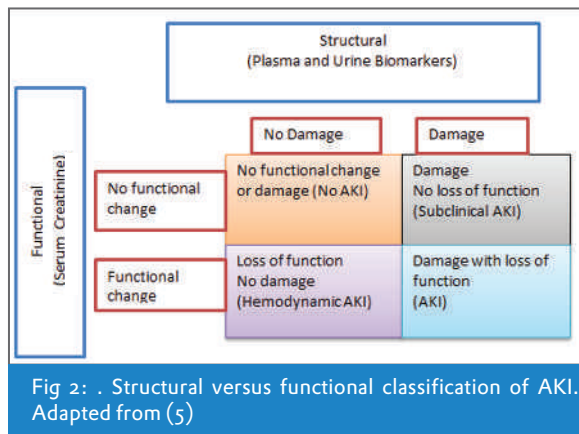


Fig 2: . Structural versus functional classification of AKI. Adapted from (5)

Irrespective of the type of insult, an inflammatory response found to play a critical role in the pathophysiology of AKI. Endothelial inflammatory injury leads to increased vascular permeability facilitating the migration of neutrophils and leucocytes into the renal interstitium and tubular lumen. Neutrophils release pro-inflammatory cytokines during transmigration which further aggravates tubular injury. Loss of cytoskeletal integrity occurs as a result of tubular response leading to desquamation of cells, apoptosis and necrosis.<sup>5</sup> The different pathophysiological events result in accumulation of various biomarkers in plasma and urine. (Fig 1) Functional and structural biomarkers of AKI. The limitation of serum creatinine in early diagnosis of AKI has lead to number of researches to identify better biomarkers in the last decade.<sup>9</sup> Biomarkers have improved the understanding of the pathophysiology associated with AKI apart from early diagnosis and risk stratification. Functional use of new acute kidney injury biomarkers (Fig 2)

### Biomarkers of acute kidney injury

An ideal AKI biomarker should be of the following characteristics.

1. Rapidly increases in the urine or blood after an insult
2. Should be highly reliable, sensitive and specific to injury.
3. There should not be any interference due to drugs or endogenous substance.<sup>10</sup>
4. It should remain elevated as long as the renal injury persists and should correlate quantitatively with the extent of renal injury.
5. It should decrease proportionately with the renal recovery status.
6. It should be nonexpensive.<sup>11</sup>

Novel biomarkers vary in their source, distribution, function and time of release after injury.<sup>6</sup> The additional benefit of novel biomarkers is that they provide prognostic information. Various biomarkers used in AKI are mentioned in Table 1. A subgroup of patients do not have AKI as defined by elevated serum creatinine but they have elevated biomarkers of tubular injury which groups them as 'subclinical AKI'. Another subgroup of population has AKI as defined by serum creatinine but with low tubular injury markers characterised as 'hemodynamic AKI'.<sup>3</sup> Based on this new concept, modification recommended in KDIGO criteria Figure 3<sup>6</sup>

Biomarker types	Biomarkers
Low molecular weight proteins	Urine CyC
Upregulated proteins	NGAL, KIM-1, L-FABP, IL-18
Functional markers	Serum creatinine, plasma/serum CyC
Enzymes	NAG, GGT, AP, $\alpha$ -GST, $\pi$ -GST

Table 1: Biomarkers of AKI

### Markers of kidney injury

**Cystatin C :** Cystatin C is a cysteine protease inhibitor produced by nucleated cells. Cystatin C is released into plasma at a constant rate, circulates in plasma and freely filtered through glomerulus. It is reabsorbed completely by endocytosis at proximal tubule. Cystatin C elevation occurs 12-24 hours post kidney damage.<sup>6</sup>

**$\beta_2$  -microglobulins:**  $\beta_2$  -microglobulins are low molecular weight glycoproteins.  $\beta_2$  Microglobulins are found on the cell surface of all nucleated cells usually filtered by glomerulus and almost completely reabsorbed and catabolised by proximal tubular cells.<sup>4</sup>

Functional criteria and/or Injury criteria	
AKIN – 1 Or RIFLE - R	Biomarker positivity (+)
AKIN – 2 Or RIFLE - I	Biomarker positivity (++)
AKIN – 3 Or RIFLE - F	Biomarker positivity (+++)

Fig. 3 – New criteria for the diagnosis of acute kidney injury. From: Acute Dialysis Quality Initiative (ADQI)

**Kidney Injury Molecule-1(KIM-1) :** KIM 1 is a type 1 cell membrane glycoprotein whose mRNA levels rise more than any other gene after kidney injury. During the injury, KIM-1 can facilitate remodelling of the injured epithelium. It is measured in urine. Features that make KIM-1 an appealing biomarker of kidney damage are lack of its expression in normal kidneys, markedly increased expression and insertion of KIM-1 into the apical membrane of the proximal tubule and persistence until the full recovery of epithelial cells.KIM-1 levels appear 12-24 hours post kidney damage.<sup>4,6,10</sup>

**IL-18** : IL-18 is a proinflammatory cytokine that is produced at the proximal tubular level. It mediates inflammatory process during sepsis, ischemia, and nephrotoxic injury. IL-18 peak occurs within 6 hours after injury.<sup>4,6</sup>

#### Neutrophil gelatinase - associated lipocalin (NGAL) :

NGAL also known as human neutrophil lipocalin or lipocalin 2 is one of the most dependable biomarker found in acute kidney injury and most extensively studied biomarker. NGAL is filtered by the glomerulus and completely reabsorbed by megalin mediated endocytosis by normal proximal tubular cells. Tubular epithelial cells produce monomeric and heterodimeric forms while activated neutrophils produce homodimeric form. Studies have shown that up regulation of NGAL gene occurs very early following kidney injury. Levels increase 2-4 hours post kidney damage. It has prognostic significance.<sup>4,6,10,11</sup>

**N-acetyl- $\beta$ -glucosaminidase (NAG)** : NAG a lysosomal enzyme is a large sized protein (>130 kDa) which originates in the lysosomes of proximal tubular cells. Due to its high molecular weight, glomerular filtration is prevented and high urinary levels are unlikely from non-renal source. Increased NAG levels reflect tubular injury and is histologically correlated with proximal tubule damage. Increased NAG levels can be seen without tubular cell injury due to increased lysosomal activity.<sup>2,5,6</sup>

#### Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) & Insulin - like growth factorbinding protein 7 (IGFBP7)

These are inducers of G1 cell-cycle arrest TIMP-2 (Urine tissue inhibitor of metalloproteinase -2) and IGFBP7 (Insulin like growth factor-binding protein 7) are the markers of cell cycle arrest which may signal stress of renal epithelium.

### Methods to Quantitate Biomarkers

There should be a standardisation in the measurement of biomarkers. Substrate based colorimetric assays followed by measurement using spectrophotometer was a traditional method used to quantitate urinary enzymes. Later ELISA became the assay of choice which detects antigen by using two epitopically distinct antibodies. Recently microbead based assay for measuring urinary KIM-1 and NGAL with multiplexing capability is in use. This technique can be used to assay cytokines. Currently a more sensitive technology which requires only one antibody and readout within minutes has been developed and on evaluation called multiplexed electrical detection nanowire sensor assay.<sup>1</sup>

### Clinical utility of biomarkers

Cystatin C is shown to better predict glomerular function in patients with chronic kidney disease and cardiac surgery even before the elevation of serum creatinine. A study conducted by Herrero-Morin et al. in critically ill children showed that cystatin C and  $\beta_2$  -microglobulins were comparatively better markers than serum creatinine for detecting GFR.<sup>4</sup>

$\beta_2$  Microglobulin is found to be elevated 4-5 days before rise in serum creatinine in situations such as renal transplantation, nephrotoxic injury, cardiac surgery. Han et al proposes that urine KIM-1 has better accuracy in identifying established kidney injury compared to early prediction of acute kidney injury.<sup>13</sup> In renal transplant patients, raised urinary KIM-1 levels may foretell the graft loss.<sup>11</sup> IL-18 can be used as a marker of proximal tubular damage in acute tubular necrosis. Studies show raised urinary IL-18 as predictor of AKI in patients with ARDS.<sup>10</sup> IL-18 is a target for biomarker therapy of AKI. Based on animal models of AKI, targeted therapies to disrupt IL-18 signalling axis could prove efficacious in attenuating renal injury. IL-18 peak occurs within 6 hours after injury indicating that Anti-IL-18 treatment need to be initiated in the first 6 hours of renal injury as urine IL-18 raises within 1st 6 hours and does not peak until 12-18 hours of renal injury.<sup>14</sup> KIM-1 and IL-18 have shown better mortality risk prediction following AKI.<sup>13</sup> Plasma and urinary NGAL have shown potential excellence as early biomarkers following cardiopulmonary bypass surgery, kidney transplantation.<sup>4</sup> Study done by Begshaw and colleagues found higher level of NGAL in septic AKI as compared to non-septic AKI.<sup>15</sup> TRIBE-AKI (Translational Research Investigating Biomarker Endpoints in AKI), was a cohort study on patients who underwent cardiac surgery found that the levels of tubular injury biomarkers like NGAL, KIM-1, L-FABP and urinary IL-18 were associated with adverse outcome.<sup>3</sup> Data from SAPPHERE study found that biomarker combinations in concert with clinical models would improve diagnostic performance of AKI biomarkers.<sup>15</sup> Currently AKI alert system or AKI sniffer in ICUs is on evaluation for usefulness in early detection and intervention in AKI.<sup>16</sup>

### Limitations

A major limitation of biomarkers is that individually they display low sensitivity and specificity. To achieve a better and early diagnosis of AKI is to combine various biomarkers.<sup>4</sup> Biomarkers in clinical practice shows promising role in children as compared to adults due to comorbidities. Limitations of various biomarkers have been listed in Table 2.

Biomarker	Limitation
Cystatin C	Levels altered in abnormal thyroid function and immunosuppressive therapy <sup>2</sup>
$\beta_2$ - Microglobulin	Rapidly degrades due to changes in room temperature and change in urine pH<6.0 <sup>1</sup>
IL-18	Levels vary in conditions such as sepsis and other inflammatory conditions
NGAL	Raised in urinary tract infections, in patients with underlying CKD, malignancies, bacterial infection, following PCI <sup>10</sup>

Table 2: Limitations of various biomarkers

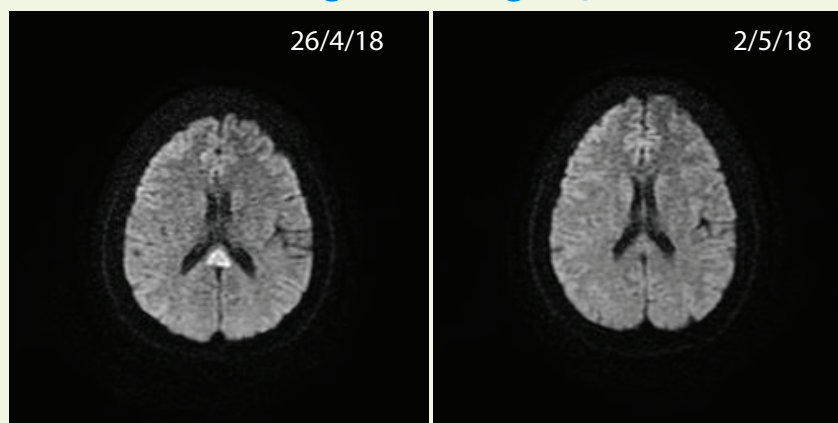
## Conclusion

The AKI pathogenesis is very complex and diverse with high mortality. Several biomarkers have been investigated over the past decade many of which have shown promising role in early detection of AKI. Biomarkers differentiate functional and structural kidney injury compared to traditional markers. Further the studies state that combining biomarkers increases the predictive value as compared to use of single biomarker. Research should be extended further to evaluate biomarkers specific to subtypes of AKI. The task now is to identify and utilize the ideal biomarker. Several new biomarkers and those still on pipeline and technologies like AKI sniffer, AKI prognosis can be improved.

## References

- 1) Vaidya VS, Ferguson MA, Bonventre JV. Biomarkers of Acute Kidney Injury. *Annu Rev Pharmacol Toxicol.* 2008;48:463-93.
- 2) de Geus HR, Betjes MG, Bakker J. Biomarkers for the prediction of acute kidney injury: a narrative review on current status and future challenges. *Clin Kidney J.* 2012;5(2):102-08.
- 3) Schaub JA, Parikh CR. Biomarkers of acute kidney injury and associations with short- and long-term outcomes. *F1000Res.* 2016;5:F1000 Faculty Rev-986.
- 4) Honore PM, Jacobs R, Joannes-Boyau O, Verfaillie L, De Regt J, Van Gorp V. Biomarkers for early diagnosis of AKI in the ICU: ready for prime time use at the bedside? *Ann Intensive Care.* 2012;2(1):24.
- 5) Martensson J, Martling CR, Bell M. Novel biomarkers of acute kidney injury and failure: clinical applicability. *Br J Anaesth.* 2012;109(6):843-50.
- 6) Lombardia F, Muryan A, Canzonieri R, Trimarchia H. Biomarkers in acute kidney injury: Evidence or paradigm? *Nefrologia* 2016;36(4):339-46.
- 7) Basile DP, Anderson MD, Sutton TA. Pathophysiology of Acute Kidney Injury. *Comprehensive Physiology.* 2012;2(2):1303-53.
- 8) Schrier RW, Wang W, Poole B, Mitra A. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest.* 2004;114(1):5-14.
- 9) Huen SC, Parikh CR. Molecular phenotyping of clinical AKI with novel urinary biomarkers. *Am J Physiol Renal Physiol. - Renal Physiology.* 2015;309(5):F406-13.
- 10) Peres LAB, Cunha Jr ADD, Schafer AJ, Silva ALD, Gaspar AD, Scarpari DF et al. Biomarkers of acute kidney injury. *J Bras Nefrol.* 2013;35(3):229-36.
- 11) Zheng CM, Liao MT, Lin MY. Biomarkers in Acute Kidney Injury. *Open Journal of Nephrology,* 2013; 3: 51-60.
- 12) Koyner JL, Parikh CR. Clinical utility of biomarkers of AKI in cardiac surgery and critical illness. *Clin J Am Soc Nephrol.* 2013;8(6):1034-42.
- 13) Coca SG, Yalavarthy R, Concato J, Parikh CR. Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney Int.* 2008;73(9):1008-16.
- 14) Alge JL, Arthur JM. Biomarkers of AKI: a review of mechanistic relevance and potential therapeutic implications. *Clin J Am Soc Nephrol.* 2015 7;10(1):147-55.
- 15) Murray PT, Mehta RL, Shaw A, et al. Current Use of Biomarkers in Acute Kidney Injury: Report and Summary of Recommendations from the 10th Acute Dialysis Quality Initiative Consensus Conference. *Kidney international.* 2014;85(3):513-21.
- 16) Colpaert K, Hoste EA, Steurbaut K, Benoit D, Van Hoecke S, De Turck F, Decruyenaere J. Impact of real-time electronic alerting of acute kidney injury on therapeutic intervention and progression of RIFLE class. *Crit Care Med.* 2012;40(4):1164-70.

## Image Challenge - 3



Clue : H/O Giddiness, Hemisensory loss, ataxia. Reversed within 1 week of treatment

- Answer in page : 64