# Emerging biomarkers for Acute Kidney Injury

Early diagnosis of AKI currently depends on detection of reduced kidney function by the rise in serum creatinine concentration, an unreliable measure in the acute setting. This article discusses the pros and cons of emerging early biomarkers which may prove useful.

by Dr Prasad Devarajan

The incidence of acute kidney injury (AKI), previously referred to as acute renal failure, has reached epidemic proportions world-wide, affecting about 7% of hospitalised patients. In the critical care setting, the prevalence of AKI requiring dialysis is about 6%, with a mortality rate exceeding 60% [1, 2]. A significant increase in morbidity and mortality associated with AKI has been demonstrated in a wide variety of clinical situations, including exposure to radiocontrast dye, cardiopulmonary bypass, mechanical ventilation and sepsis. Once established, the treatment is largely supportive, at an annual cost surpassing $10 billion in the US alone.

The early diagnosis of AKI currently depends on detection of reduced kidney function by the rise in serum creatinine concentration, which is a delayed and unreliable measure in the acute setting. In general, there are several non-renal factors influencing the serum creatinine concentration such as body weight, muscle mass, race, age, gender, total body volume, drugs, muscle metabolism and protein intake. In the face of AKI, serum creatinine is an even poorer reflection of kidney function, because the subjects are not in steady state, and serum creatinine therefore lags far behind renal injury. Furthermore, significant chronic kidney disease can exist with minimal or no change in creatinine because of renal reserve and enhanced tubular secretion of creatinine. Ironically, experimental studies have identified interventions that may prevent or treat AKI if instituted early in the disease process, well before the serum creatinine rises [2, 3]. The lack of predictive biomarkers has impaired our ability to translate these promising findings to human AKI. A troponin-like biomarker of AKI that is easily measured, unaffected by other biological variables and capable of both early detection and risk stratification would represent a tremendous advance in clinical medicine [4].

The search for AKI biomarkers is an area of intense contemporary research. Conventional urinary biomarkers such as casts and fractional excretion of sodium are insensitive and non-specific for the early recognition of AKI. Other traditional urinary biomarkers such as filtered high molecular weight proteins and tubular proteins or enzymes have also suffered from lack of specificity and dearth of standardised assays. Fortunately, the application of innovative technologies such as functional genomics and proteomics to human and animal models of AKI has uncovered several novel genes and gene products that are emerging as biomarkers. The most promising of these are listed in Table 1, and detailed in this article.

## Neutrophil gelatinase-associated lipocalin (NGAL)

Preclinical studies in animal models identified NGAL (also known as lipocalin 2) as one of the most upregulated genes and proteins in the kidney very early in the course of AKI. Following the serendipitous finding that NGAL protein in animals was easily detected in the urine soon after AKI, a number of translational studies have now established NGAL as an early diagnostic biomarker for AKI in common human clinical situations. In prospective studies of children who underwent elective cardiac surgery, AKI (defined as a 50% increase in serum creatinine) occurred 2-3 days after surgery [5]. In contrast, NGAL levels in urine

| Biomarker name | Sample source | Cardiopulmonary bypass (CPB) | Contrast administration | Critical care setting | Kidney transplant (tx) | Commercial assay |
|---|---|---|---|---|---|---|
| NGAL | Urine | <2 h post-CPB | 2 h post-contrast | 48 h pre-AKI | 12-24 h post-tx | ELISA, ARCHITECT# |
| IL-18 | Urine | 6 h post CPB | Not increased | 48 h pre-AKI | 12-24 h post-tx | ELISA |
| KIM-1 | Urine | 12 h post CPB | Not tested | Not tested | Not tested | ELISA |
| L-FABP | Urine | 4 h post-CPB | 24 h post-contrast | Not tested | Not tested | ELISA |
| NL | Plasma | <2 h post-CPB | 2 h post-contrast | 48 h pre-AKI | Not tested | ELISA, Triage* |

Table 1. Novel biomarkers for the early prediction of AKI in humans. AKI: acute kidney injury, defined as a 50% increase in serum creatinine from baseline; NGAL: neutrophil gelatinase-associated lipocalin; IL-18: interleukin 18; KIM-1: kidney injury molecule 1; L-FABP: liver-type fatty acid binding protein. The times indicated (in hours) refer to the earliest time points at which the biomarker is increased significantly from baseline. #The ARCHITECT Assay is manufactured by Abbott Diagnostics. * The Triage NGAL Test is manufactured by Biosite Inc.

and plasma measured by ELISA revealed a dramatic increase, within two hours of the surgery, in those who subsequently developed AKI. Both urine and plasma NGAL were excellent independent predictors of AKI, with an area under the curve for the receiver-operating characteristic (AUC-ROC) of > 0.9 for the 2-6 hour measurements [5]. These findings have now been confirmed and published in several additional prospective studies of adults and children who developed AKI after defined clinical events such as cardiac surgery (10 studies), contrast administration (four studies) and kidney transplantation (two studies). Urine and plasma NGAL have also emerged as robust biomarkers that predict development of AKI in heterogeneous groups of patients with unknown timing of kidney injury, such as subjects admitted to emergency or critical care settings (six published studies). A collective analysis of all the published performances of NGAL as a predictive biomarker for AKI to date [reviewed in 2-4] reveals a mean AUC-ROC of 0.82 (95% confidence interval 0.7-0.9). The mean derived sensitivity and specificity from these studies are in the 75-80% range. From the published literature, the derived optimal cut-off value of NGAL for the early prediction of AKI appears to be at a median value of approximately 150 ng/mL.

NGAL is also emerging as an early biomarker in interventional trials. For example, a reduction in urine NGAL has been employed as an outcome variable in clinical trials demonstrating the improved efficacy of a modern hydroxyethyl-starch preparation compared with albumin or gelatin for maintaining renal function in cardiac surgery patients. Similarly, the response of urine NGAL was attenuated in cardiac surgery patients, who experienced a lower incidence of AKI after sodium bicarbonate therapy as compared to sodium chloride. Furthermore, subjects who developed AKI after aprotinin use during cardiac surgery displayed a dramatic rise in urine NGAL in the immediate post-operative period, attesting to the potential use of NGAL for the prediction of nephrotoxic AKI.

Recent studies have also demonstrated the utility of early NGAL measurements for predicting clinical outcomes of AKI. In subjects undergoing cardiac surgery, the two hour post-operative plasma NGAL levels were strongly correlated with duration and severity of AKI and length of hospital stay. In addition, the 12 hour plasma NGAL was strongly correlated with mortality [6]. Similarly, the two hour urine NGAL levels were strongly correlated with duration and severity of AKI, length of hospital stay, dialysis requirement, and death [7]. A collective analysis of 10 published studies of NGAL as a predictive biomarker for dialysis requirement reveals a mean AUC-ROC of 0.78 (95% confidence interval 0.69-0.87).

The NGAL results described thus far have been obtained using research-based ELISA assays, which are impractical in the clinical setting. A major recent advance from the clinical laboratory perspective has been the development of a point-of-care kit for the clinical measurement of plasma NGAL (Triage NGAL Device, Biosite Incorporated). The assay is user-friendly, with quantitative results available in 15 minutes, and requires only microlitre quantities of whole blood or plasma. In subjects undergoing cardiac surgery, the two hour plasma NGAL measurement measured by the Triage Device showed an AUC of 0.96, sensitivity of 0.84, and specificity of 0.94 for prediction of AKI using a cutoff value of 150 ng/mL [6]. Furthermore, a urine NGAL immunoassay has also been developed for a standardised clinical platform (ARCHITECT analyser, Abbott Diagnostics). This assay is also easy to perform; no manual pretreatment steps are necessary, a first result is available within 35 minutes and only 150 microlitres of urine are required. Following cardiac surgery, the two hour urine NGAL measurement by ARCHITECT analyser showed an AUC of 0.95, sensitivity of 0.79, and specificity of 0.92 for prediction of AKI using a cutoff value of 150 mg/mL [7]. Both clinical platforms are CE-marked for the European Union, and are undergoing multi-centre validation studies.

Clearly NGAL represents a novel predictive biomarker for AKI and its outcomes. However, there are some limitations [2-4]. Plasma NGAL measurements may be influenced by coexisting variables such as chronic kidney disease (CKD), chronic hypertension, systemic infections, inflammatory conditions and malignancies. Urine NGAL levels are also elevated in subjects with CKD, lupus nephritis and urinary tract infections. However, the levels of both plasma and urine NGAL in these clinical situations are significantly lower than those typically measured in AKI. These issues are therefore unlikely to impact on the clinical utility of NGAL as an excellent biomarker for AKI.

## Interleukin 18 (IL-18)

IL-18 is a pro-inflammatory cytokine that is known to be induced and cleaved in the proximal tubule, and subsequently can be easily detected in the urine following ischaemic AKI in animal models. In an initial cross-sectional study, urine IL-18 levels measured by a commercially available ELISA were markedly elevated in patients with established AKI, but not in subjects with urinary tract infection, chronic kidney disease, nephrotic syndrome or prerenal azotaemia. In a subsequent study of patients who developed AKI two to three days after cardiac surgery, urinary IL-18 levels peaked at 12 hours post surgery, and predicted AKI with an AUC-ROC of 0.75 [8]. Urine IL-18 is also considered an early biomarker of AKI in the intensive care setting, being able to predict this complication about two days prior to the rise in serum creatinine. Early elevated urine IL-18 levels correlated with the severity of AKI as well as mortality.

Thus, IL-18 may also represent a promising biomarker for AKI. IL-18 is more specific to ischaemic AKI, and levels are largely unaffected by nephrotoxins, chronic kidney disease or urinary tract infections. However, IL-18 measurements may be influenced by a

number of coexisting variables, since renal IL-18 mRNA levels are known to be induced in other disease states such as endotoxaemia, immunological injury and cisplatin toxicity. Furthermore, plasma IL-18 levels are known to be increased in various systemic inflammatory states, and the relationships between plasma and urine IL-18 remain unexplored.

## Kidney injury molecule 1 (KIM-1)

In animal models, kidney injury molecule 1 is one of the most highly induced proteins in the kidney after AKI, and a proteolytically processed ectodomain of KIM-1 is easily detected in the urine soon after AKI. Assays for KIM-1 (ELISA and microbead-based) have been developed in research laboratories, but are not commercially available. In a small human cross-sectional study, KIM-1 was found to be markedly induced in proximal tubules from kidney biopsies in patients with established AKI, and urinary KIM-1 measured by ELISA distinguished ischaemic AKI from prerenal azotaemia and chronic renal disease. In a case-control study of children undergoing cardiac surgery, urinary KIM-1 levels were enhanced in subjects who developed AKI, with an AUC of 0.83 at the 12 hour time point [9]. In a larger prospective cohorts study of hospitalised patients with established AKI, urinary KIM-1 was associated with adverse clinical outcomes, including dialysis requirement and death.

Thus KIM-1 may represent a promising AKI biomarker. An advantage of KIM-1 as a urinary biomarker is the fact that its expression seems to be limited to the injured or diseased kidney, and no systemic source of KIM-1 has been described. However, urinary KIM-1 measurements may be influenced by a number of other confounding variables. KIM-1 is induced in the kidney and upregulated in the urine by a large number of nephrotoxins, including cyclosporine, cisplatin, cadmium, gentamicin, mercury and chromium. Similarly, KIM-1 in the kidney and urine is induced in a variety of chronic proteinuric, inflammatory and fibrotic disease states in humans.

## Liver fatty acid binding protein (L-FABP)

Liver fatty acid binding protein (L-FABP) is a 14 kDa protein normally expressed in the proximal convoluted and straight tubules of the kidney, and upregulated in animal models of AKI. An ELISA for this analyte is commercially available; it was first used to demonstrate that urinary L-FABP levels were significantly increased prior to the increase in serum creatinine in those patients who developed AKI post contrast dye. In a recent prospective study of children undergoing cardiac surgery, urine L-FABP increased at four hours post-bypass, with an AUC of 0.810 for a cutoff value of 486 ng/mg creatinine [10].

Thus, L-FABP also appears to represent a promising AKI biomarker. However, urinary L-FABP measurements may also be influenced by a number of confounding variables. Several studies have documented increased urinary L-FABP levels in patients with non-diabetic chronic kidney disease, early diabetic nephropathy, idiopathic focal glomerulosclerosis and polycystic kidney disease. Additionally, L-FABP is also abundantly expressed in the liver, and urinary L-FABP may be influenced by serum L-FABP levels.

## Summary

Of the AKI markers discussed in this article, NGAL has entered the final phases of the biomarker development process, facilitated by the development of commercial tools for its measurement in large populations across different laboratories. But will any single biomarker such as NGAL suffice for AKI management? In addition to early diagnosis and prediction, it would be desirable to identify biomarkers capable of identifying aetiologies, predicting clinical outcomes, allowing for risk stratification and monitoring the response to interventions. In order to obtain all of this desired information, a panel of validated tandem biomarkers may be needed, with temporal profiles as illustrated in Figure 1. However, one must also take into account the technical and cost issues surrounding the identification, validation, commercial development and acceptance of multi-marker panels. From the recent cardiology literature, we can see that troponin as a stand-alone biomarker provides excellent diagnostic and prognostic information in acute coronary syndromes

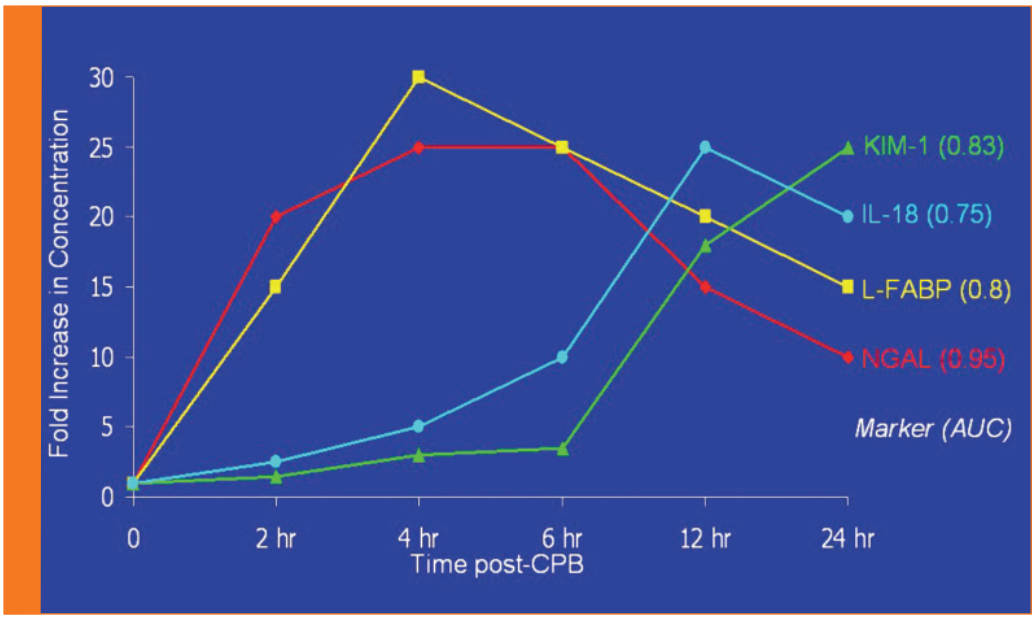


Figure 1. Urinary biomarker profiles in subjects who develop AKI after surgery with cardiopulmonary bypass (CPB). AKI: acute kidney injury, defined as a 50% increase in serum creatinine from baseline; NGAL: neutrophil gelatinase-associated lipocalin (data from reference 5); IL-18: interleukin 18 (from reference 8); KIM-1: kidney injury molecule 1 (from reference 9); L-FABP: liver-type fatty acid binding protein (from reference 10).

and acute decompensated heart failure. If the current prospective multicentre studies measuring NGAL levels using standardised laboratory platforms provide promising results, we may already have the "AKI troponin".

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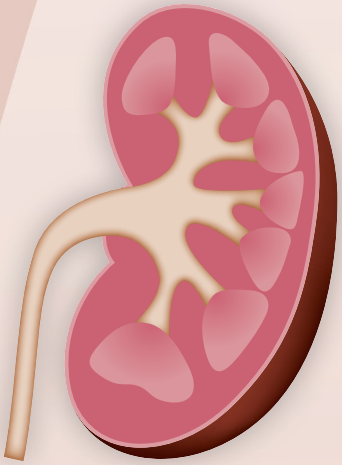

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