

# Biomarkers and the Clinical Nephrologist

By Jennifer R. Charlton and Mark D. Okusa

Clinicians view kidney disease as a continuum where kidney failure results from a combination of patient susceptibility factors (diabetes, hypertension, or low nephron mass) combined with episodes of kidney injury (acute kidney injury [AKI]). Clinicians use traditional biomarkers such as serum creatinine, urine output, and albumin as indices of kidney function to diagnose, prognosticate, implement therapy, and monitor progression. These traditional biomarkers are far from ideal. Serum creatinine is a surrogate for kidney function, not injury, and often only signals the injury after several days. Creatinine is also a poor surrogate for renal reserve in assessing patients for chronic kidney disease (CKD) as more than 50 percent of a patient's nephrons have to be nonfunctional before it will increase. Urine output is hindered by diuretic use, inaccurate collection, and lack of specificity (1). There are many etiologies that lead to renal disease and complex compartments within the kidney that can be injured (vasculature, interstitium, glomeruli, and tubules). These factors make the development of specific biomarkers and the interpretation of these markers particularly challenging, but nonetheless critically important to assist the clinical nephrologist.

## Biomarkers in AKI

Clinicians are challenged to recognize early and identify quickly the underlying causes of AKI in order to implement the appropriate therapies that may reduce the risk of progressive kidney disease. For example, many biomarkers have been tested to determine if they can distinguish prerenal AKI from intrinsic AKI with the latter due to tubule injury from medications, sepsis, or ischemic injury (2). During clinical AKI, there are alterations in the renal microcirculation and tissue oxygenation that ultimately lead to early cellular injury when cells release these markers into the plasma or urine which provide a window into the local environment of the kidney (1). Therefore, the baseline state of the kidney and the cumulative damage leading up to the episode of AKI play a role in the response to injury (1) and affect how we interpret these biomarkers.

Promising diagnostic markers of renal function and damage include cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), and liver fatty acid binding protein (L-FABP). Each of these markers provides information regarding a different part of the nephron (3). Cystatin C is a small protein produced by all cells that is freely filtered and degraded by the proximal tubule and has become a clinically utilized biomarker. Also with glomerular injury and a disruption of the filtration barrier, albumin is excreted in the urine. When there is a tubular injury both albumin and cystatin C are not reabsorbed and are excreted in the urine. There are also markers that are released from damaged cells (N-Acetyl- $\beta$ -D-glucosaminidase [NAG],  $\alpha$  glutathione S-transferase [GST],  $\pi$ -GST, and collagen IV) and those that increase in response to damage (NGAL, KIM-1, L-FABP, and IL-18). NGAL is an iron-carrying protein that has been extensively studied with many attractive traits as a biomarker for AKI. It increases quickly within hours of renal injury and is both sensitive and specific. KIM-1 is also a promising marker for the detection of AKI as it is produced during proximal tubule injury and may be particularly useful in determining drug toxicity. These

biomarkers open the door to patients with "subclinical AKI" who have undetectable changes in creatinine, but an increase in biomarkers reflecting kidney damage (3,7). In the current state of development, novel biomarkers must be interpreted with caution as not all perform well in every AKI setting (2).

Just as there are various segments of each nephron subject to injury, there are various causes of renal injury. Some injuries—such as cardiac surgery, contrast nephropathy, and nephrotoxins—have a known time of exposure, making clinical studies more straightforward. In other clinical scenarios, such as sepsis and hepatorenal disease, the time course and the primary injury can be obscured.

Beyond predicting the development of AKI, some biomarkers have been able to prognosticate severity and duration of AKI, likelihood for renal replacement therapy, and nonrecovery of function and death (4). These predictions appear to be stronger in the population where the timing of the renal insult is known (cardiac surgery) (4).

An ongoing dilemma in renal biomarker research is what should be the gold standard for the validation of these novel biomarkers. Just demonstrating the superiority to serum creatinine or urine output is a flawed approach and correlation between biomarkers and histologic damage does not occur in clinical trials. Therefore, the outcomes in AKI will not likely change until we gauge a biomarker's worth by its ability to provide a trigger for a clinical action (initiate or monitor therapy) (4). The association between these biomarkers and clinically relevant outcomes is needed (3).

There are still significant challenges in AKI biomarker research. First, we need to address patients with underlying CKD or in a setting where the renal health of the patient is not known prior to AKI (2). Second, we have to assess the usefulness of a biomarker to predict the progression to CKD as the time course may be long and variable. Finally, cutoffs for various markers, bedside utility, platform standardization, interlaboratory calibration (4), and the cost-benefit ratio of these biomarkers are all areas to address.

## Biomarkers in CKD

Nephrologists require improved tools for the early diagnosis of the 26 million adult Americans who suffer from CKD and are at risk for developing ESRD (5). The most commonly utilized and validated biomarkers in CKD are eGFR and proteinuria. Similarly to AKI biomarkers, these biomarkers are retrospective or insensitive. Novel biomarkers are being studied in tubulointerstitial injury, glomerular injury, endothelial dysfunction, oxidative stress, inflammation, and fibrosis. As it is becoming more recognized that AKI is a prelude to CKD, the biomarkers assessed in AKI are also being tested in the setting of CKD (5).

Cystatin C,  $\beta$ -trace protein (BTP), and uric acid have been used as surrogates for kidney function, but require more rigorous validation in larger populations and are not being used in clinical practice (5). In clinical studies, NGAL concentration correlated to CKD staging, validated in various etiologies of CKD, and predicted kidney function decline. Other tubulointerstitial markers—such as KIM-1, NAG, and L-FABP—still need long-term studies in larger populations to ensure their validity as markers in CKD (5). Newer biomarkers sensitive to glomerular injury, such

as nephrin, podocin, and podocalyxin, have been assessed in lupus, postinfectious, and IgA nephritis, and in their early stages seem to be specific to glomerular diseases (5). Other markers, such as C-reactive protein and IL-18, markers of inflammation and fibrosis, are being assessed in progressive kidney disease (5). Specific pathophysiologic mechanisms of primary renal diseases leading to CKD and elements of CKD progression are shared by all forms of progressive CKD, thus assessing both nonspecific and disease-specific markers is needed.

## Biomarkers in transplantation

There is a large effort to investigate novel biomarkers that could guide titration of immunosuppressive medications tailored to the biologic suitability of the graft and recipient (6). Studies have unmasked a genetic signature that was highly sensitive and specific in patients experiencing chronic renal allograft rejection or tolerance (6). Additionally, urine proteomic analysis can distinguish chronic allograft injury from healthy controls and those patients with excellent graft function (6). Although promising, this area of research needs significantly more validation in a prospective fashion with larger cohorts of patients with attention to graft protection (6).

In summary, there is an intensive effort to develop novel biomarkers of kidney disease as currently used clinical biomarkers have shortcomings. There is hope, however, for implementing point-of-care use of panels of biomarkers in the near future. For the clinician, such tools will assist in therapy and counseling of their patients with the goal of improving outcomes. ●

*Jennifer R. Charlton, MD, is an Assistant Professor of Pediatrics, Division of Pediatric Nephrology, and Mark D. Okusa, MD, FASN, is the JoÛ C. Buchanan Distinguished Professor of Medicine at the University of Virginia Health System in Charlottesville, VA.*

## References

- Okusa MD, et al. Physiological biomarkers of acute kidney injury: A conceptual approach to improving outcomes. *Contrib Nephrol* 2013; 182:65–81.
- Endre ZH, et al. Differential diagnosis of AKI in clinical practice by functional and damage biomarkers: Workgroup statements from the tenth acute dialysis quality initiative consensus conference. *Contrib Nephrol* 2013; 182:30–44.
- McCullough PA, et al. Implementation of novel biomarkers in the diagnosis, prognosis, and management of acute kidney injury: Executive summary from the tenth consensus conference of the acute dialysis quality initiative (ADQI). *Contrib Nephrol* 2013; 182:5–12.
- Cruz DN, et al. Use of biomarkers to assess prognosis and guide management of patients with acute kidney injury. *Contrib Nephrol* 2013; 182:45–64.
- Fassett RG, et al. Biomarkers in chronic kidney disease: A review. *Kidney Int* 2011; 80:806–821.
- Bestard O, et al. Biomarkers in renal transplantation. *Curr Opin Organ Transplant* 2010; 15:467–473.
- Charlton JR, et al. A basic science view of acute kidney injury biomarkers. *Nephrol Dial Transplant* 2014 Jan 2 [Epub ahead of print]