Biomarkers of Acute Kidney Injury: Dawning of a New Era
By Joseph Bonventre

The kidney community has devoted a great deal of effort to building consensus regarding the definition of acute kidney injury (AKI). This has resulted in RIFLE classification and AKIN network (AKIN) criteria focused on changes in serum creatinine (SCr) and rate of urine production. These changes in SCr are important and have been shown to be predictive of outcome in a number of studies. SCr changes, however, can be affected by a large number of things unrelated to kidney injury, including drug interference with secretion of creatinine into the tubule, muscle mass, gender, age, and renal reserve, a measure of how much the kidney can compensate for injury.

Kidney injury, which leads to a reduction in GFR, is not immediately followed by an increase in SCr. This lag time greatly impedes the early diagnosis of AKI, delays therapy, and hinders the ability to test new therapies early in the course of the disease when AKI is much more likely to be amenable to interventions that might alter its natural history. SCr concentration can also increase due to functional decreases in GFR that are not associated with tubular injury. This is the case in prerenal azotemia. It has thus been recognized for a number of years that new biomarkers of injury would be very desirable. A “kidney troponin,” for example, would allow for the identification of true injury to the kidney as troponin does for the myocardium.

A biomarker is defined as a characteristic that can be objectively measured and evaluated as an indicator of normal biologic or pathogenic processes of pharmacologic responses to a therapeutic intervention (1). Examples of biomarkers are proteins; lipids; genomic, metabolic, or proteomic patterns; imaging determinations; electrical signals; and cells present in a urinalysis. Having a biomarker that directly reflects injury and is easily measured from fluid that is easily obtained, such as blood or urine, would introduce a new paradigm in which we would be directly monitoring injury rather than a secondary consequence of injury—a reduction in GFR, as manifest by an increase in SCr.

In commenting on a major initiative of the FDA that focuses on biomarkers, Janet Woodcock, MD, deputy commissioner for operations and head of FDA’s Critical Path Initiative, said: “Most researchers agree that a new generation of predictive biomarkers would dramatically improve the efficiency of product development, help identify safety problems before a product is on the market (and even before it is tested in humans), and facilitate the development of new types of clinical trials that will produce better data faster” (2). The FDA has provided guidance that a biomarker can be considered “valid” if (i) it is measured in an analytical test system with well established performance characteristics and (ii) there is an established scientific framework or body of evidence that elucidates the physiologic, pharmacologic, toxicologic, or clinical significance of the test result (3).

We need better biomarkers to diagnose AKI earlier, to predict outcome in a patient with AKI with standard therapy, and to identify who will respond to an intervention and whether the intervention is working. In addition, better biomarkers will permit better stratification of patients for clinical trials and potentially lead to definition of new therapeutic targets for AKI. A good predictive biomarker will have a significant effect on evaluation of potential therapies because it will enable the identification of subgroups of patients who will have a high incidence of kidney injury and hence reduce the number of patients needed to study in order to test potential therapeutic strategies.

A clinically useful new kidney biomarker will improve the sensitivity and specificity for the detection of renal injury and discriminate renal injury, which may have long-term consequences, from adaptive responses that may be reflected by transient BUN/creatinine increases that return to pre-elevated levels over time, even with continued exposure to the agent in question. It is also likely that some of these biomarkers will be useful not only for AKI, but also to monitor severity and progression of glomerular or tubular-interstitial disease in patients with chronic kidney disease.

Over the years, a large number of biomarkers of kidney injury have been suggested, yet for various reasons, none have been routinely accepted in animal or clinical studies. In some cases, the biomarker was felt to be too sensitive, not sensitive enough, or too nonspecific. In other cases, the biomarker was unstable with storage. More recently, however, there have been a number of advances in the application of biomarkers to AKI.

Promising biomarkers
A limited number of biomarkers are currently being evaluated by a number of groups, and clinically useful reagents have been identified. Some of the promising urinary biomarkers for AKI include: microalbuminuria, kidney injury molecule-1 (KIM-1), N-acetyl ß-D glucosaminidase (NAG), neutrophil gelatinase associated lipocalin (NGAL), cystatin C, L-fatty acid binding protein (L-FABP), and interleukin-18 (4).

Biomarkers that can be measured in blood or urine in both experimental animals and humans are of particular interest. It may be possible to draw on the experimental information obtained for such biomarkers in animals to help guide the use and interpretation of biomarker studies in humans. Biomarkers that have been well studied and characterized as very sensitive biomarkers of injury in animals, if they function similarly in man, may make it possible to monitor safety and efficacy in clinical trials when the ability to obtain kidney tissue is severely constrained and when the severity of the injury early on is insufficient to result in an increase in SCr. Blood and urine are two convenient fluids in which to measure a particular biomarker of kidney injury. Urine has the advantage of being readily available noninvasively and amenable to straightforward testing by both health care professionals and patients themselves. Given its normally low protein content, there is also less interaction in the biomarker assay with proteins. On the other hand, large variations in physical chemical properties of urine may affect reliability of the test for the biomarker and/or stability of the analyte.

Given the importance to the clinical, pharmaceutical, and regulatory communities motivated by early intervention and safer therapies, there has been a great deal of activity devoted to examining the role of various potential biomarkers of kidney injury in both animals and humans. Biomarkers have been proposed to reflect injury to various parts of the nephron or to reflect interstitial disease (5), although in many cases the specific type of nephron damage has not been sufficiently studied.

Ischemia/reperfusion injury and most tubular toxins have as their primary site of injury the proximal tubule. If in some cases the primary site of injury is more distal along the nephron, the proximal tubule is often also secondarily involved. Although there are some important exceptions to this generalization, such as lithium, whose toxicity is predominantly distal nephron-related, in general a biomarker sensitive for proximal injury will be useful for many clinical scenarios as well as very useful in safety monitoring and assessment.

Kidney biomarkers in drug approval
The importance of the identification of kidney injury biomarkers for patient safety was manifest quite clearly in July 2008 when the FDA and the European Medicines Agency (EMA) agreed to accept data for seven kidney toxicity biomarkers as part of the drug approval process. This was the first time that a single application was submitted to both regulatory agencies.
The recognition of the inadequacy of kidney injury biomarkers that have been used for 50–100 years (BUN and creatinine) is telling us. Point-of-care technologies, such as dipsticks, will be very useful in making the biomarkers more available for use in rat studies. The PSTC is currently working to identify sufficient evidence for the FDA and EMEA to qualify some or all of these markers for human studies.

Much of the development of biomarkers has grown out of kidney safety and drug toxicity studies. The information obtained from these studies will go far to inform the use of these biomarkers—and potentially others—for patients with sepsis, ischemia, and other drug and nondrug-related forms of injury. Understanding the performance of kidney injury biomarkers, however, is much more straightforward in animals than it is in humans. In animals there is a very good “gold standard”: renal pathology. In humans, pathology is infrequently available.

Novel approaches must be developed because comparisons to SCr are not satisfactory for the reasons already mentioned. A change in SCr, especially if it is transient and reasonably modest, does not necessarily imply kidney injury. A biomarker may be increased without a change in SCr but that does not impugn the biomarker necessarily since there may be significant injury that is not sufficient to produce an increase in SCr. On the other hand, SCr may be increased and a biomarker not increased when there is no injury but rather a hemodynamic change that results in an elevation in SCr.

There is a strong tendency in the growing literature on this topic to compare biomarkers to SCr as a gold standard. Under certain circumstances SCr is a very reasonable metric since it provides insight into GFR; however, the inadequacies of SCr as a gold standard represent barriers to understanding of the true performance of the biomarker to diagnose injury. Patient outcome is a “hard endpoint.” Patients are quite complicated, however, with many things contributing to long-term outcome. It has been suggested that multiple biomarkers will be more useful than one and this, I believe, may be true if we really understand what each biomarker is telling us. Point-of-care technologies, including dipsticks, will be very useful in making the biomarkers more available for routine clinical use.

The recognition of the inadequacy of kidney injury biomarkers that have been used for 50–100 years (BUN and creatinine) has led to intense interest in finding and validating new biomarkers. New biomarkers will enable us to diagnose kidney injury earlier and provide better information about the status of ongoing injury in patients with chronic kidney disease. This will add to the armamentarium of personalized medicine by better informing interventional, diagnostic, and therapeutic decision-making to minimize kidney injury and optimize interventional strategies. I am convinced that better kidney injury biomarkers will provide us with better tools that will result in better outcomes for our patients.

### References