

The role of novel 'leaky' and 'up-regulated' biomarkers of kidney injury

Serum creatinine, the universal 'gold standard' measure of kidney injury, is a measurement of the functional capacity of the kidney. The unit of function in the kidney is the nephron, containing a glomerulus and an elongated convoluted tubule. Each kidney has over one million nephrons and they can be envisioned as pumping waste from the plasma. They have a high capacity such that 60% of nephrons can be physically damaged before serum creatinine levels are elevated; the remaining intact nephrons simply increase their 'filtration' capacity. In terms of what an ideal biomarker may contribute to predicting kidney injury, creatinine rises much too late. Serum creatinine is renal medicine's 'flawed gold standard'.

In contrast to the functional biomarker of serum creatinine, injury in other organs is often assessed by use of constitutive 'leaky' biomarkers. Clinical biochemistry has long been comfortable with the concept of biomarker proteins specific for different cell types and tissues, which 'leak' from those cells when injured. These are necrotic markers which appear in body fluid only when the integrity of the cell membrane is damaged by toxicity, ischemia or disease.

Examples of commonly used constitutive biomarkers are:

- Heart: The troponins, myoglobin and creatine kinase are constitutively expressed in cardiac myocytes and are released when these cells are damaged, usually by ischemia. They 'leak' into blood when their source cells are damaged and are accepted as the 'standard of care' for myocardial injury.
- Liver: Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are accepted Standards of liver injury, 'leaking' into plasma when hepatocyte injury occurs.
- Pancreas: Serum amylase and lipase, which exist in high quantities in pancreatic acinar cells, 'leak' into urine and plasma when these cells are physically damaged.

All of these markers can be referred to as constitutive 'leaky' biomarkers. They are the 'gold standard' of tissue injury and exist pre-formed in large concentrations in their respective cells. This makes them ideal biomarkers as they 'leak' rapidly when the cell membrane of their source is physically disrupted and their concentrations are related to the extent of injury.

If the 'Gold Standards' of other organs can be used as a model for the kidney then cellular specific markers which 'leak' when source cells are damaged may be a distinct improvement on serum creatinine. The ideal kidney biomarker might then be thought of as a molecule which can be measured in urine, which detects physical damage to different parts of the nephron early, and whose concentration is related to the level of injury.

The glomerulus is primarily involved in filtration but much of the damage that occurs in the kidney is tubular. Along the length of the tubule, different parts carry out different functional work and are injured differentially by different drugs and disease events. A combination of constitutive 'leaky' biomarkers which can 'walk down' the nephron would ensure that all tubular injury can be detected. The biomarker(s) should tell one that damage is occurring long before serum creatinine rises. In fact

Ideally the biomarker will predict a subsequent rise in serum creatinine, and when this damage is arrested the marker levels should reduce rapidly.

A panel of constitutive histologically specific markers for both the rat and human kidney has been assembled. They leak into urine when their source cells are damaged and predict a subsequent rise in serum creatinine if the insult continues. Their concentrations are related to the level of injury and they can be used to stage the degree of renal injury; recently Pi Glutathione-S-Transferase (GST) has been shown to be a very sensitive predictor of Stage III Acute Kidney Injury (AKI) post coronary bypass and is elevated in a significant number of type II diabetics.

	Human	Rat
Proximal Tubule	Alpha GST	Alpha GST
Distal Tubule	Pi GST	GST Yb1
Collecting Duct	HCD	RPA-1

A second class of kidney biomarkers that have received much study in recent years are the ‘up-regulated’ biomarkers. Research using RNA analysis has identified a number of genes which are significantly up-regulated in different parts of the nephron in response to injury. When stressed, tubular cells focus their biochemical resources to the location of the insult to prevent necrosis. The up-regulated proteins are secreted to influence neighboring cells and to ameliorate apoptosis, the first response to an insult. The up-regulated markers may well be able to save the target cell before necrosis occurs, thus they do not necessarily reflect necrosis. The two leading markers in this category are Kidney Injury Molecule 1 (KIM-1) and Neutrophil Gelatinase Associated Lipocalin (NGAL).

KIM-1 is found specifically in the proximal tubule and is a well documented early marker of proximal tubule injury. It does not detect distal tubule or collecting duct damage. It has been reported as elevated in the urine of Type II diabetes whereas the ‘leaky’ proximal tubule biomarker, Alpha GST is only seen intermittently in this group.. KIM-1 may therefore be reflecting the proximal tubule’s response to a diabetes related insult but the physical damage is elsewhere (in this case it appears to be distal tubule in nature, with significant elevations in Pi GST, the ‘leaky’ distal tubule marker.) Urinary N-Acetyl-Glucosaminidase (NAG), Interleukin 18 (IL-18) and Liver Fatty Acid Binding Protein (L-FABP) are also known to be up-regulated in the proximal tubules upon insult.

NGAL is one of the most up-regulated gene products in the kidney in the course of AKI. It is distributed throughout the kidney, is up-regulated in a range of conditions and is a useful diagnostic.

From a clinical perspective a physician may not be interested in mechanisms, but to know that all tubular damage, which will eventually lead to an elevation in serum creatinine, can be detected early enough to treat usefully. From a toxicologists viewpoint known mechanisms of response to injury and the use of translational markers may be valuable. There is much discussion in the field of nephrology concerning the value of panels of kidney biomarkers. An appropriate mix of markers (both ‘leaky’ and ‘up-regulated’) may well allow for optimum diagnosis of injury.