Mini-Review

New Markers of Acute Kidney Injury: Giant Leaps and Baby Steps

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Abstract
Treatment of acute kidney injury has been hampered by the inability of a creatinine-based diagnosis to allow clinicians to intervene with timely treatments aimed at preventing further development of the disease to the point where renal replacement therapy is necessary or death occurs. Novel biomarkers of injury have been touted as the tool by which early detection can occur and, on that basis, novel treatments can be developed and delivered early in the disease process. Sufficient new biomarkers have been discovered and evaluated to expect that not one biomarker but a panel of biomarkers applied according to phase of injury, baseline renal function and comorbidities will be necessary for the early diagnosis of acute kidney injury. Issues of validation of these biomarkers remain, particularly in heterogeneous populations of critically ill patients. Nevertheless, we are rapidly moving towards an era where the diagnosis of acute kidney injury will be proactive rather than by the traditional diagnosis of exclusion.

Introduction
Acute Renal Failure was re-designated Acute Kidney Injury (AKI) because the common, frequently fatal, rapid loss of filtration in critically ill patients is precipitated by significant tubular or glomerular injury. Because of their association with poor outcomes, the functional decrease in glomerular filtration rate (GFR) and its surrogates, plasma creatinine increase or urine output decrease, remain the consensus definition of AKI. However, the search for sensitive and specific biomarkers of such injury has become a major focus of the academic nephrology community.[1] Driving this search is the hope that early detection of AKI will lead to development of successful early intervention strategies.[2] The present delay in detection has contributed to the failure of interventions designed to treat AKI which have been successful under experimental conditions. This delay can be attributed to our reliance on the use of serum creatinine on which to base intervention decisions, despite knowing that creatinine is a surrogate marker of GFR, not tubular injury. While GFR and serum creatinine are inversely related, extrapolation to GFR from serum creatinine is not appropriate except under steady-state conditions. The half-life of creatinine with a normal GFR (120 mL/min) is approximately 4 hours. Consequently, following a 50% reduction in GFR (doubling the half-life), the three to five half lives required to reach a new steady-state take 24 to 40 hours. Typically diagnosis is 24 to 72 hours following the event that precipitated the reduction in GFR, long past the therapeutic window of opportunity. A new paradigm of treatment of AKI is needed in which diagnosis is possible soon after injury, the phase of AKI is identified, and intervention is tailored appropriately (Figure).

Giant Leaps: New Discoveries
Discovery of biomarkers of renal cellular injury and the re-discovery of the relationship between enzymuria and renal injury offer hope that this paradigm will now change.[3-5] These biomarkers are present in urine, plasma and sometimes both. The urinary biomarkers fall into at least five categories: filtered biomarkers where glomerular permeability has increased, filtered biomarkers where renal reabsorption is impaired as a result of tubular injury, preformed tubule cellular biomarkers released following injury, up-regulated biomarkers, and activated biomarkers released into the urine following recruitment of inflammatory cells. Table 1 lists the most studied biomarkers according to these categories. More detail on these and other biomarkers useful in detecting cellular injury has been the subject of other reviews.[6,7] At present, the most studied biomarkers are tubular enzymes GGT, glutathione S transferase (GST), and N-acetyl-beta-D-glucosaminidase (NAG), and the more recently discovered induced biomarkers, Kidney Injury Molecule 1 (KIM-1), Interleukin 18 (IL-18) and Neutrophil Gelatinase Associated Lipocalin (NGAL).[8-10] New biomarkers are appearing regularly in the literature including Trefoil factor 3,[11] Clusterin,[12]

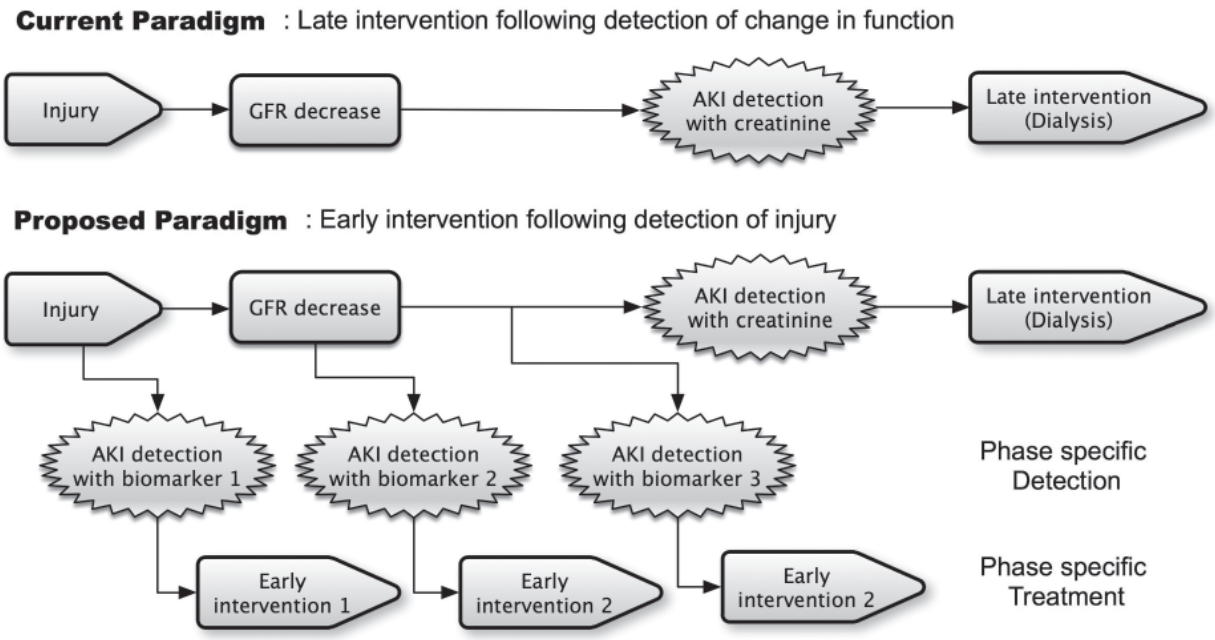

Figure. The promise of early detection and early treatment by phase specific biomarkers.

Table 1. Novel urinary biomarkers of AKI.

Biomarker	Type	Description
Albumin	Filtered- a. Glomerular b. Impaired tubular absorption	Normal serum constituent
Aprotinin	Filtered	Exogenous
Cystatin C	Filtered- Impaired tubular absorption	Normal serum constituent
β2-microglobulin	Filtered- Impaired tubular absorption	Normal serum constituent
Liver Fatty Acid Binding Protein (L-FABP; FABP1)	Pre-formed and Filtered- Impaired tubular absorption	Proximal convoluted tubule cytoplasm
Alkaline Phosphatase (ALP)	Pre-formed	Proximal tubule brush border
γ-glutamyl transpeptidase (GGT)	Pre-formed	Proximal tubule brush border
α-glutathione S transferase (α-GST)	Pre-formed	Proximal tubule cells
π-glutathione S transferase (π-GST)	Pre-formed	Distal tubule cells
N-acetyl-beta-D-glucosaminidase (NAG)	Pre-formed	Proximal tubule lysosomes
Clusterin	Up-regulated	Proximal tubules in outer medulla (pre-formed in distal tubule)
Interleukin 18 (IL-18)	Up-regulated in proximal epithelial cells and recruited macrophages	Pro-inflammatory cytokine
Kidney Injury Molecule 1 (KIM-1)	Up-regulated	Proximal tubule cells
Netrin-1	Up-regulated	Tubular epithelial cells
Neutrophil Gelatinase Associated Lipocalin (NGAL)	Up-regulated	Distal tubule cells (absorbed in proximal tubule)
Trefoil factor 3 (TFF3)	Down-regulated	Proximal tubules in outer medulla

Netrin-1[13] and Aprotinin.[14] Albumin may have a specific role as a biomarker in AKI since proximal tubular receptor-mediated uptake may be impaired.[11,15]

Commercialisation of the NGAL assay has facilitated the wide study of this particular biomarker to the point where meta-analysis has been done.[16] With the increasing availability of these biomarkers, the question arises as to how and when they might replace serum creatinine in the diagnosis of AKI. There is little doubt that they detect injury earlier than creatinine detects functional loss. However, for most, the time course and relationship to renal injury is poorly understood. Definition of their behaviour is limited to homogeneous populations, usually following specific and well-timed injury, such as following cardiopulmonary bypass or exposure to nephrotoxins such as iodinated contrast. Furthermore, the primary tool for assessing the value of these injury biomarkers has been to assess their ability to prognose increases in serum creatinine as a surrogate for changes in GFR. However, serum creatinine changes in the critically ill are poorly related to function. Furthermore, the metric of interest is change from a normal baseline serum creatinine which is often unknown, and current methods to estimate it from anthropomorphic data lead to errors in diagnosis.[17] In order to be useful for screening patients, these biomarkers require validation in heterogeneous populations as well as fulfilling a number of requirements summarised in Table 2.

Standardisation is facilitated by the commercialisation of these assays. A proliferation of studies may be expected for NGAL as a result of the commercial availability of both urinary and plasma assays. The relationship of biomarker concentration to time and type of injury is essential for characterising possible damage. While this is intuitive, it is clear that because the different biomarkers have different profiles after injury, a panel of biomarkers will be required for accurate timing and identification of the phase of injury.[2] Recent data demonstrating that biomarker performance is modified by baseline renal function further complicates the design and interpretation of an effective biomarker panel.[18] The effects of co-morbidity on novel biomarker performance has yet to be studied in depth although there is evidence that sepsis may be a confounding factor.[15] The role of each biomarker in the causal pathway of injury, or the post-causal pathway leading to other organ damage needs to be carefully defined, and this too is available only for a limited number of biomarkers such as KIM-1, IL-18, NGAL and cystatin C. There are few studies of biomarker performance in heterogeneous populations and fewer where these have been undertaken prospectively. Biomarker performance is almost inevitably poorer in heterogeneous than in the homogeneous populations where the biomarker was first validated.

Baby Steps Towards a New Treatment Paradigm

None of the caveats discussed is insurmountable. The first trial of a novel therapy for AKI, high dose erythropoietin (EPO), has been conducted following the elevation of a novel biomarker of injury (the EarlyARF trial).[19] The trial successfully triaged 163 critically ill patients to an intervention arm of a randomised control trial on the basis of a raised urinary GGT and ALP expressed as a product. Important lessons were learnt, including the importance of utilising a biomarker whose period of elevation following a renal insult coincided with the timing of sampling. In practice it is expected that there will be needed a panel of biomarkers which not only gives details on the location and type of injury (Table 1), but also on the phase of development of injury from early onset to late functional change. The panel will also need to reflect the severity of injury, just as creatinine increase reflects the severity of functional change. It is likely the ideal panel will vary according to the clinical scenario. A panel for cardiac surgery may consist of only one or two biomarkers able to detect ischaemic-reperfusion injury shortly after bypass, whereas a panel for use in intensive care will need biomarkers to detect injury from a variety of causes, from one hour to several days post injury, and in the presence of

Table 2: Biomarker validation requirements.

Establishment of how each biomarker is: • related to time and type of injury • correlated with base-line renal function • modified by co-morbidities
Establish each biomarker's role in renal causal injury pathway
Validate each biomarker against hard outcomes*
Validate biomarker panels against hard outcomes*
Develop point-of-care or rapid assays for each biomarker to allow timely intervention

*need for renal replacement therapy and short- and long-term mortality.

other organ damage, systemic inflammation and sepsis. When therapies target one type and phase of injury, such as EPO targeting apoptosis, biomarker panels will need to distinguish between the type and phase of injury.

Conclusion

We are transiting a period where these biomarkers must be validated against the less than satisfactory gold-standard of serum creatinine, itself a surrogate for the hard outcome of renal 'failure' requiring dialysis and the even harder outcome of mortality.

Nevertheless, biomarker research is progressing rapidly. After detection in experimental studies, and demonstration of clinical utility, further studies in experimental models are required to determine the phase specificity of each biomarker in relation to injury. The utility of each biomarker in assessing recovery from injury after therapeutic intervention also needs to be undertaken and these phase-specific biomarkers will then need to be re-validated in clinical studies. The proliferation of biomarker studies suggests that we are rapidly moving towards an era where the diagnosis of acute kidney injury will be proactive rather than the traditional diagnosis of exclusion.

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