Novel biomarkers of acute kidney injury: time for implementation?

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Acute kidney injury (AKI) is associated with significant early and late [1] mortality, even after 10 years and regardless of the degree of functional recovery [2]. After AKI, 7.8 subjects per 100 patient years develop chronic kidney disease and 4.9 per 100 patient years proceed to end-stage renal disease [3]. Since our demonstration in 2003 that urinary biomarkers collected on admission to an intensive care unit could predict development of AKI [4], an ever increasing number of novel biomarkers of kidney damage have emerged. Most of these novel biomarkers and the older cluster of tubular enzymes are markers of structural injury, in other words, 'renal damage biomarkers'. They have been used for AKI risk assessment, early diagnosis, and prognosis, and are potentially useful for differential diagnosis, and for monitoring the course of injury and response to therapy. This has been recognized and incorporation into definitions of AKI has been recommended by both the Acute Dialysis Quality Initiative group [5] and by regional practice guidelines in Australia and New Zealand [6].

Incorporation of damage biomarkers into clinical practice awaits agreement regarding the appropriate thresholds (cut-offs) for diagnosis or categories of severity. As noted [7], such thresholds are contextual and can vary with cause and context of AKI. As measured by the area under the receiver operator curve (AUC), biomarker performance varies considerably, particularly in heterogeneous populations [8]. However, as highlighted by Kellum and Devarajan in this issue [9], even a modestly performing biomarker with an

AUC less than 0.70 can assist in clinical decision making, simply by selecting an appropriate cutoff. Given the poor utility of serum creatinine in AKI, they argue further that it is already imperative that these biomarkers are used in clinical trials to enrich the target population for interventions, with the obvious critically needed benefit of reducing the number needing to be enrolled. Clearly, the same considerations apply whether triaging with biomarkers is used to identify AKI or recovery from AKI [10].

Biomarker threshold selection requires assay standardization and identification of normal reference intervals. Commercialization helps drive assay standardization and assays for the best performed novel biomarkers, neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) are widely available. Only one published study has examined the effects of different storage conditions on urinary biomarker concentrations [11]. Reference intervals for novel biomarkers are not widely available and current trials have used different strategies for defining these after the fact. For instance, we demonstrated that subjects with so-called 'pre-renal' AKI had mildly elevated urinary KIM-1, IL-18 and cystatin C compared with subjects who did not develop an increase in serum creatinine at any time [12]. These observations were confirmed by Doi *et al.* [13] who observed increases in urinary L-type fatty acid binding protein (L-FABP), NGAL, IL-18, *N*-acetyl- β -D-glucosaminidase (NAG) and albumin in similarly defined subjects in an intensive

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care unit. These observations confirm that transient increases in serum creatinine in critically ill subjects are associated with increases in damage biomarkers and highlight that prerenal AKI is merely the mild end of a continuous spectrum of severity of AKI.

Up to now, biomarker cutoffs have been validated against serum creatinine, since absolute or relative increases in creatinine are the primary basis for the consensus definitions of AKI, although the changes actually are surrogates for change in GFR [14]. While a biomarker increase can predict an increase in creatinine, only the eventual change in creatinine can diagnose AKI. This circular argument ignores the well-known deficiencies of creatinine and suggests further that only changes in function are important. Hence an alternative GFR surrogate to serum creatinine, such as serum cystatin C might be substituted and damage biomarker prediction assessed against cystatin C based diagnosis of AKI. This too is illogical.

The beauty of the matrix of damage and function markers recommended for diagnosis by the ADQI group [5] is that either damage or function marker change or both can be used for diagnosis. However for this to be useful, the cutoffs for functional and damage markers in the ADQI AKI matrix need to be derived against an independent gold standard. Since dialysis requirement or death are significant clinical outcomes of AKI, we proposed a method for establishing biomarker cutoffs by selecting values that had the same sensitivity for these outcomes as serum creatinine [15]. This method was examined in a total of 507 subjects and generated values for absolute urinary biomarker concentrations that are similar to those revealed for NGAL by a metaanalysis of observational studies in cardiorenal AKI [16] and for both NGAL and KIM-1 in a large cohort screened in the emergency department [17]. While the values require validation in large cohorts, the method is robust and will allow classification, staging and differential diagnosis of AKI using the damage function matrix recommended by ADQI. This or similar approaches that define cut-offs independent of serum creatinine are needed for the prospective validation of the biomarker-positive, creatinine-negative category of AKI and to allow evolution to injury biomarker-based triaging of patients outside the domain of clinical research.

One study has highlighted the GFR dependence of plasma NGAL levels [18]. In this issue, McWilliam *et al.* [19] report reference intervals for urinary NGAL and KIM-1 in healthy children in both United States and United Kingdom. They identified that NGAL was consistently higher in females than males and ethnic differences and diurnal variation in urinary KIM-1 concentrations. Studies also reported in this issue by

Mohammadjafari [20] demonstrate that in neonates with an antenatal diagnosis of hydronephrosis, urinary KIM-1 and epidermal growth factor levels identify those with severe obstruction. A study in Poland [21] has demonstrated that male, obese, hypertensive adolescents with hyperuricemia have higher urine NGAL and KIM-1 levels relative to normouricemia adolescents. Taken together, these studies highlight the urgent need to define urinary and plasma damage biomarker reference intervals in diverse healthy populations.

Variation in biomarker performance nevertheless allows room for even newer biomarkers that might perform better in both selective and heterogeneous populations. Biomarkers that are highly selective for target populations are needed to facilitate individualization of patient care: so-called 'theragnostics' discussed briefly by Kellum and Devarajan in this issue [9]. On the other hand, identification of discriminating biomarkers from heterogeneous (instead of homogenous) populations leads to novel biomarkers that can be used more widely in less well-defined subjects. For example, the excellent performance of new urinary biomarkers, the cell cycle inhibitors, namely, tissue inhibitor of matrix metalloproteinase, TIMP-2, and insulin-like growth factor-binding protein, IGF7BP7, has been validated in the heterogeneous setting of critically ill patients [22]. These more recently identified biomarkers of damage may be better performed because they are more sensitive and less selective and thus respond to a wider variety of renal insults than earlier novel biomarkers selected on the presumed basis of a single mechanism of renal injury.

New novel biomarkers, new uses for existing markers and ways of identifying further biomarkers are discussed in several papers in this issue. Haase *et al.* recently used an antibody-free proteomic approach to identify three novel biomarkers of kidney function, fibrosis and ischemic tissue damage, namely, LG3 (a C-terminal fragment of perlecan), latent transforming growth factor binding protein-2, LTBP-2 (an extracellular matrix protein) and cathepsin L, a ubiquitously expressed cysteine endopeptidase. In the present study, Haase *et al.* [23] use MS analysis to demonstrate in patients undergoing open heart surgery, that at differing times, LG3, LTPBP2, cathepsin L as well as NGAL and cystatin C had greater predictive value for AKI and major adverse kidney events than creatinine, urea and urine output. Fernando *et al.* [24] review calcineurin inhibitor (CNI) metabolism and discuss how various methods, particularly Omics strategies, have revealed potential biomarkers that might distinguish CNI nephrotoxicity from rejection or ischemic injury in renal transplant recipients. Che *et al.* [25] demonstrate that mildly reduced serum bicarbonate, both

alone or in combination with serum creatinine predict poorer long-term renal function after an episode of AKI. Finally, because of the importance of catecholamine metabolism in the setting of cardiac surgery, Albert *et al.* [26] examined the association between genotypes of catecholamine-O-methyltransferase and AKI in 200 adult patients undergoing cardiopulmonary bypass; importantly the authors utilized both serum creatinine and the urinary damage biomarkers NGAL and midkine to test these associations.

We are fortunate to be in the biomarker era. After decades of delay and uncertainty in diagnosis of AKI, there now exist methods for early detection, differential diagnosis and for monitoring renal status that are independent of serum creatinine. This collection of papers addresses critical issues in discovery and implementation of renal biomarkers in clinical practice and research. While optimization and cost remain ongoing concerns, the most salient conclusion from these

studies, is that, we can no longer afford not to use biomarkers in risk stratification for clinical trials. Furthermore, we need to take further note of the lessons learned by our colleagues in cardiology, who have focused on ‘how’ not ‘whether’ to use damage biomarkers in myocardial ischemic injury. Selected damage biomarkers should be starting to enter routine clinical practice in parallel with functional markers wherever possible.

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