Using Biomarkers for Acute Kidney Injury: Barriers and Solutions

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Key Words

Biomarker · Acute kidney injury · Cutoff

Abstract

The clinical implementation of urinary and plasma renal injury biomarkers has been hampered by the variability associated with nonstandardized commercially available biomarker assays, uncertainty and variations in patient selection criteria, and the absence of context-specific cutoffs for biomarker concentrations. These limitations are increased by comparison with serum creatinine to define acute kidney injury. The critical problem affecting biomarker performance is patient heterogeneity involving the cause, context (including comorbidity and baseline renal function), and timing of the injury. We suggest strategies for stratifying subjects to provide appropriate context, and illustrate a creatinine-independent method for defining thresholds for biomarker concentrations in these contexts which utilizes the same sensitivity for the clinical outcomes of dialysis or death. Large multicenter cohort studies are needed to validate the proposed cutoffs.

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Introduction

Novel biomarkers outperform serum creatinine in the early diagnosis of acute kidney injury (AKI). Despite widespread enthusiasm from nephrologists, intensivists, and cardiologists, the implementation of urinary and plasma renal injury biomarkers has been hampered by the variability associated with nonstandardized commercially available biomarker assays, uncertainty and variations in patient selection criteria, and the absence of context-specific cutoffs for biomarker concentrations. Fundamental to these concerns is the absence of a genuine gold standard against which to evaluate these cutoffs, since comparison with creatinine represents a circular argument if creatinine is also used to define the outcome. Despite such limitations, the Acute Dialysis Quality Initiative (ADQI) has recommended redefining AKI diagnosis and staging in terms of both damage and functional markers [1, 2]. We have suggested strategies for stratifying subjects to provide an appropriate context and methods of defining thresholds for biomarker concentrations in these contexts. Large multicenter cohorts will be needed to validate the proposed cutoffs. The critical problem affecting biomarker performance is patient heterogeneity involving the cause, context (including comorbidity and baseline renal function), and timing of the injury. Solutions to these barriers are discussed below.

As we have highlighted previously [3], the initial biomarker performance is usually outstanding because the biomarkers are selected on the basis of relatively homogeneous cohorts that differ only in terms of the presence or absence of AKI. The biomarkers are selected after receiver operating characteristic analysis yields an area under the curve (AUC) well above 0.90. Such preliminary studies are followed by similarly carefully selected homogeneous validation cohorts with similarly excellent biomarker performance results. However, then the biomarker is applied to less homogeneous patient groups in necessarily more heterogeneous cohorts (so-called phase 3 studies), and when these include high-risk patients as well as affected patients the biomarker performance is automatically degraded. In many studies, the outstanding performance of novel biomarkers with initial AUCs above 0.90 becomes of questionable utility, with AUCs frequently below 0.70. Examples of performance degradation abound not only for older biomarkers such as tubular enzyuria, e.g. γ -transpeptidase [4] versus the results of Endre et al. [5], but also for novel biomarkers, e.g. neutrophil gelatinase-associated lipocalin (NGAL) and liver fatty acid-binding lipoprotein [6] versus the results of Siew et al. [7].

The critical factor in the degradation of biomarker performance is heterogeneity among the patients undergoing assessment [3]. This heterogeneity may involve the duration of renal injury prior to biomarker measurement or of baseline renal function prior to injury. Stratification by either or preferably both of these parameters improves biomarker performance [5]. Heterogeneity may involve the cause of AKI (e.g. very diverse causes such as sepsis vs. ischemia-reperfusion injury) [3]. Heterogeneity of the cause may simply involve the inclusion of mild (low-risk) versus moderate-to-severe (high-risk) injury secondary to the same cause, such as so-called 'prerenal' versus established (intrinsic) hypoperfusion AKI [8], where what is actually a continuum of injury appears discontinuous because of differing selection criteria such as early reversibility or response to fluid therapy, although these criteria really just represent severity criteria [9]. Heterogeneity of comorbidity may similarly play a role in degrading the performance of individual biomarkers. This may be the explanation for the differing performance of urinary versus plasma NGAL in differing contexts. For example, among adults and children immediately after cardiopulmonary bypass surgery, plasma but not urinary NGAL was predictive of AKI in the adult population [10] while urinary but not plasma NGAL was predictive in children [11]. Nevertheless, in both populations the AUCs were

modest (in the mid 0.70 range), and quintile comparisons rather than preselected cutoffs were used to draw conclusions about performance.

So, what strategies can we use to improve biomarker performance? Careful patient selection, timed selection of specific biomarkers, and serial biomarker measurement, perhaps in a panel of different biomarkers, are useful future strategies.

Careful patient selection for some of these parameters will prospectively improve the biomarker performance. Provided the biomarker time course is known, it is theoretically possible to select ideal biomarker(s) when the injury is reasonably well timed. Thus, the immediate answer in cardiopulmonary bypass appears obvious from the example above. However, even this context remains inadequately defined since significant biomarker dilution is likely in the immediate postbypass setting. An analogous setting is cardiac arrest. We combined creatinine and volume kinetics to account for fluid type, and rates of fluid infusion and urine output to analyze the effect of dilution on biomarker (serum creatinine) profiles in 49 patients after cardiac arrest (mostly out-of-hospital arrest) [12]. A reduction in creatinine production was required to account for the degree of decrease in serum creatinine observed in the majority of patients in this setting. More importantly, only a decrease in the glomerular filtration rate (GFR) of approximately 50% could account for maintenance of a normal serum creatinine in 6 patients, while an immediate and progressive increase was only observed in 2 patients. These results strongly support the use of fluid balance to correct serum creatinine and avoid a misdiagnosis of AKI, as advocated by Macedo et al. [13]. There is no reason to assume that other plasma biomarkers, including NGAL, are not similarly diluted. We would also advocate for correction for dilution of both functional and structural biomarkers in the analysis of postoperative settings associated with significant fluid therapy.

Thus, timing and correction for dilution may enhance the biomarker performance prospectively. The optimal timing is also modified by baseline renal function, as demonstrated in the EARLYARF study where the peak performance of each biomarker varied with both the duration after renal injury and the baseline GFR before renal injury [5]. While the stratification used to demonstrate performance enhancement in that study was used in retrospect, the message for prospective use is to measure the biomarkers at specific time points after injury based on both the individual biomarker profile and the baseline GFR. Such an approach lends itself to the identification

Table 1. Urinary biomarker cutoffs based on sensitivity linked to dialysis or death

	NGAL, ng/ml	KIM-1, pg/ml	IL-18, pg/ml	CysC, ng/ml	sCr, mg/dl	n
Sensitivity-linked method						
62% sensitivity for death or RRT	140	1,850	118	950	>1.3 mg/dl or >50%*	507 [18]
Emergency room screening						
Cutoff	104	2,817	65	171	1.4	
Sensitivity/specificity for AKI	68/81	52/79	43/58	46/59	81/82	1,635 [20]

RRT = Renal replacement therapy. * Above baseline within 48 h (AKIN criteria).

of a panel of biomarkers that includes one or more that increase early and others that increase later after injury. This would allow timing of the injury as well as diagnosis. An alternative strategy with a single biomarker is serial measurement. This is analogous to serial troponin measurement in the diagnosis of acute myocardial ischemic injury. At present, biomarker profiles are not well defined. However, several studies suggest that a reduction in the concentration of some injury biomarkers predicts recovery; for example, a reduction in urinary NGAL and hepatocyte growth factor on day 14 in the ATN trial predicted survival and dialysis independence at 60 days [14]. While encouraging, few serial measurement studies of urinary or plasma injury biomarkers have been performed that allow comment on clinical utility in the prediction of recovery.

Can We Select Better Biomarkers?

While maneuvers that enrich the population homogeneity yield higher biomarker AUC values for diagnosis, population enrichment is not always an option. Common clinical scenarios are examples of this. For example, heterogeneous populations are the norm in the emergency department or on admission to adult intensive care units. Thus, a realistic context is critical. The newest novel biomarkers are the cell cycle arrest markers urinary insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2), both inducers of G1 cell cycle arrest, a key mechanism implicated in AKI. In a heterogeneous cohort of 728 critically ill patients, the combination of these two biomarkers demonstrated an AUC of 0.80 (0.76 and 0.79 alone) [15]. A head-to-head comparison with all the other novel biomarkers currently available revealed that urinary [TIMP-2] · [IGFBP7] was significantly superior to all previously de-

scribed markers of AKI ($p < 0.002$), none of which achieved an AUC above 0.72. The authors also demonstrated the added value of the biomarkers by showing that [TIMP-2] · [IGFBP7] significantly improved the risk stratification when added to a 9-variable clinical model analyzed using the Cox proportional hazards model, a generalized estimation equation, and the integrated discrimination improvement or net reclassification improvement strategies. In sensitivity analyses, [TIMP-2] · [IGFBP7] remained significant and superior to all other markers regardless of changes in the reference creatinine method. While these results are exciting by any reckoning, the strategy underpinning the improved biomarker performance may be that these 2 cell cycle inhibitors were selected from over 300 biomarkers in 3 distinct cohorts including patients with sepsis, shock, major surgery, and trauma. Thus, the selection was based on performance in a heterogeneous discovery cohort rather than a homogeneous one. Presumably, this strategy created a higher initial hurdle for selection but then resulted in less degradation of the biomarker performance when tested in other similar real-world heterogeneous settings.

Patient Classification Using Biomarkers Requires Reliable Cutoffs

Up to now, these 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 [16] even when incorrectly calculated [17]. Perhaps the real reason for using creatinine is our conceptualization of the GFR as an integrated marker of renal function which has superseded urea (or BUN) as a means of identifying the need for dialysis. In any case, validation of biomarker performance against creatinine-

based definitions creates a circular argument. While a biomarker increase can predict an increase in creatinine, only the inevitably delayed change in creatinine can diagnose AKI. This further ignores that creatinine is a marker of function, and actually a continuous variable, while injury biomarkers detect structural damage, which is arguably a dichotomous variable (present or absent). This also ignores the frequently recited deficiencies of serum creatinine [16]. Thus for validity, the cutoffs for functional and damage markers in the ADQI AKI matrix need to be derived against an independent gold standard. Unlike preclinical studies, renal histology is not routinely available as the gold standard. Since dialysis requirement and 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 [18]. This method was examined in a discovery cohort (n = 253) and then assessed in a validation cohort (n = 254). The method generated values (table 1) for absolute urinary biomarker concentrations that were similar to those revealed for NGAL by a meta-analysis of observational studies in cardiorenal AKI [19] and for both NGAL and KIM-1 in a large cohort

screened in the emergency department [20]. While the absolute 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 the ADQI. This or similar approaches that define cutoffs independently of serum creatinine will allow prospective validation of the biomarker-positive, creatinine-negative category of AKI which is needed for application of the ADQI proposals and will finally allow evolution to injury biomarker-based triaging of patients outside of the domain of clinical research.

Conclusions

- Biomarker performance is critically affected by heterogeneity of the cause, context, baseline renal function, and timing of the injury.
- Biomarker cutoffs need to be assessed against outcomes independently of serum creatinine.
- Sensitivity for dialysis or death can be used to identify biomarker cutoffs for AKI diagnosis.

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