Biological Markers of Acute Kidney Injury

Edward D. Siew,* Lorraine B. Ware,† and T. Alp Ikizler*

Divisions of *Nephrology and Hypertension and †Allergy, Pulmonary, and Critical Care Medicine, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee

ABSTRACT

An abrupt change in serum creatinine, the most common indicator of acute kidney injury (AKI), is strongly linked to poor outcomes across multiple clinical settings. Despite endless attempts to distill the magnitude and timing of a changing serum creatinine into a standardized metric, singular focus on this traditional functional marker obligates the characterization of AKI to remain, at best, retrospective and causally noninformative. The resultant inability to meaningfully segregate critical aspects of injury such as type, onset, propagation, and recovery from ongoing decrements in renal function has hindered successful translation of promising therapeutics. Over the past decade, however, the emerging field of clinical proteomics reinvigorates hope of identifying novel plasma and urine biomarkers to characterize cause and course of kidney injury. Efforts to validate these markers for use in clinical studies now show early promise but face important obstacles including interpretive difficulties inherent in using serum creatinine as a sole comparator for diagnostic performance, a need to better evaluate the incremental performance of new markers above established clinical and biochemical predictors, a relative lack of power to sufficiently examine hard clinical end points, and a potential over-reliance on use alone of receiver operating curves for assessing biomarker utility. Here, we discuss efforts to address these barriers and further ascertain the clinical value of new markers.


Despite evolving sophistication in hospital care, acute kidney injury (AKI) remains common and tightly associated with high morbidity and mortality.1–5 At minimum, AKI presents an array of derangements detailed by the underlying precipitant, variable anatomic sites of involvement, and the metabolic complications of unregulated inflammation,6–8 oxidative stress,9 and insulin resistance.10 As these perturbations can profoundly hinder recovery, the persistence of poor outcomes is not entirely surprising.11,12 Yet despite the multifaceted nature of AKI, retrospective estimation of a single aspect of kidney function using dichotomized changes in a single marker (i.e. serum creatine), remains the gold standard for phenotyping this complex disease.13

Serum creatinine distinguishes AKI from other acute illnesses including sepsis, myocardial infarction, or the acute respiratory distress syndrome in which both indicators of injury (blood/urine cultures, troponin elevation, or abnormal chest x-ray) and functional markers (WBC count, electrocardiogram abnormalities, or oxygenation) fulfill diagnostic criteria.14,15 Contrasted with the evolution of therapeutic options in these classical syndromes, translation of effective interventions targeting AKI is hindered both by the inherent delay and inaccuracies in traditional diagnostic approaches and the inability to discern beyond clinical impression those who might benefit from a given treatment.16,17

Emerging proteomic technology over the past decade has engendered new hope for identifying novel protein indicators to help characterize the pathophysioloogy of disease. Concurrently, the potential for improving risk stratification, informing clinical decision making, and guiding pharmaceutical development recently led the American Society of Nephrology to designate the development of novel AKI biomarkers a top research priority.18 The response over a few years resulted in the identification of nearly 20 potential markers reported in nearly 120 articles of varying quality attempting to validate the utility of markers in human AKI (Supplemental Table 1). Some of the more promising of these include either urine or plasma neutrophil gelatinase–associated lipocalin (NGAL),19,20 kidney injury molecule-1 (KIM-1),21,22 IL-18,23–25 cystatin C,25,26 liver fatty-acid binding protein (L-FABP),27 IL-6,28 α/β glutathione S-transferase (GST),29–31 and N-acetyl-β-D-glucosaminidase (NAG).32,33

This review highlights the early experience validating these markers for clinical use and discusses efforts to address common problems that have emerged.
including the use of serum creatinine as a gold standard for determining diagnostic and prognostic performance, a lack of statistical power to sufficiently examine harder outcomes and integrate known predictors of injury, over-reliance on the area under the receiver-operating curve (AUC-ROC) as a measure of biomarker performance, and potential limitations of the markers themselves. Discussion of unique aspects of biomarker discovery is beyond the scope of this brief review and can be found elsewhere.34–36

Table 1 lists the most extensively studied candidate markers for AKI in humans and includes their source (urine/plasma), cellular localization in the kidney, assay platform, and acquisition setting. The rationale for AKI biomarker candidacy derives from preclinical identification of candidate markers serving either a functional (enzymatic: NAG, α/α′-GST, and γ-glutamyl transpeptidase (GGT); inflammatory: IL-18 and NGAL) and/or structural role (KIM-1, Na+/H+ exchanger isoform 3, and L-FABP) within renal tubular epithelia, or as low molecular weight proteins normally filtered by the glomerulus and/or metabolized by healthy tubular epithelia (cystatin C, α1/B2 microglobulin, and retinol binding protein). The native functions of these markers situate them in various intracellular locations or on the plasma membrane. In commonly used animal models of AKI including ischemia-reperfusion or nephrotoxic injury, active release or shedding of these markers in either free or membrane-bound form (exosomes) into the urine after tubular damage has prompted testing in an analogous setting of human injury such as cardiopulmonary bypass. Serum/plasma markers, particularly low molecular weight proteins filtered by the kidney such as cystatin C and inflammatory markers including IL-6 and NGAL, have also been studied.

PROPOSED PHASES OF CLINICAL AKI BIOMARKER VALIDATION

The rapid proliferation of marker discovery is not unique to nephrology. In 2009, the American Heart Association released a scientific statement describing criteria for the evaluation of novel markers in human studies including six proposed phases of evaluation (Figure 1; Supplemental Table 2). Review of the biomarker literature in AKI indicates that efforts to date largely consist of small single-center studies falling within the early phases (phase 1 to 2, and to a much lesser extent, phase 3), suggesting that the validation process for the majority of renal markers remains in its infancy.

Phase 1: Established AKI

Early proof-of-concept studies involving cross-sectional examination of newly proposed markers such as urine IL-18, NGAL, L-FABP, KIM-1, α-γ-GST, NAG, and cystatin C generally demonstrate excellent ability to discriminate between carefully selected patients with established AKI and control patients (healthy individuals, hospitalized patients, or patients with chronic kidney disease) without acute elevations in serum creatinine.22,24,37–40 Subsequent prospective evaluation of these markers in more heterogeneous settings such as the emergency room or intensive care unit suggests utility for some markers such as NGAL or the NHE3 for distinguishing between those likely to have AKI and those with other causes of acute serum creatinine elevation such as prerenal azotemia.41,42

Phase 1: Early Detection

A major clinical need to identify a therapeutic window during which to apply putative interventions in those with early damage before a detectable change in function occurs has driven another important area of investigation, namely early detection of ongoing injury. In this regard, cardiopulmonary bypass (CPB), along with nephrotoxic injury and renal transplantation represent attractive venues for initial testing as timing of injury can be pinpointed and biomarker measurement planned accordingly. Furthermore, these settings often closely mimic the mechanistic model, renal artery cross-clamping, where several candidate markers were originally studied. Enthusiasm for this line of investigation has been enhanced by several small single-center studies in children undergoing CPB that reported robust separation in the levels of urine biomarkers between AKI and non-AKI patients with discriminative performance assessed by AUC-ROC values ranging between 0.75 to 0.99,20,38,43–46 Studies extended to adults undergoing CPB have had more variable results, but generally less robust predictive value for AKI, including NGAL (AUC 0.54 to 0.87),47–49 IL-18 (AUC 0.55 to 0.87),50,51 cystatin C (AUC 0.73 to 0.76),48,52,53 KIM-1 (0.68 to 0.78),38,54 and NAG (0.63).54 Similarly, urine NGAL and IL-18 in other isolated settings of renal injury, including kidney transplantation55,56 or contrast nephropathy57,58 demonstrate moderate to good discrimination. When extrapolated to predicting AKI in more heterogeneous injury settings such as the critically ill, single measurement of markers including urine NGAL,59,60 IL-18,61–63 cystatin c,64 or plasma NGAL65,66 are moderately useful, although plasma/serum cystatin c25,26,67 shows some promise in relatively larger studies. Continued examination of the early predictive capacity of candidate markers in these and other settings of injury including liver transplantation,68 sepsis,7,69–71 and various nephrotoxins72–74 remains an active area of investigation.

INTERPRETATIVE CHALLENGES

Creatinine as a Reference Standard

As specific biomarker data accumulate in different clinical settings suggesting varying degrees of discrimination or predictive ability, critical interpretative challenges have surfaced. The most apparent result from a singular focus on serum creatinine as a reference standard despite acknowledged limitations in its sensitivity and specificity. Examples include a variable delay for creatinine to increase after injury depending on renal reserve,75 the need for substantial tubular damage before creatinine elevation occurs, and independent contributors to variations in serum creatinine (pre-
<table>
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<th>Biomarker</th>
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<tr>
<td>NGAL</td>
<td>Urine/serum</td>
<td>PT &gt; DT neutrophils; other epithelial cells</td>
<td>Upregulated expression during ischemic PT injury and detectable in urine</td>
<td>Binds siderophores (iron trafficking); growth/differentiation factor</td>
<td>CPB&lt;sup&gt;b&lt;/sup&gt;; ICU&lt;sup&gt;b&lt;/sup&gt;; ER&lt;sup&gt;b&lt;/sup&gt;; contrast&lt;sup&gt;b&lt;/sup&gt;; trauma&lt;sup&gt;a&lt;/sup&gt;; liver transplant&lt;sup&gt;b&lt;/sup&gt;; HUS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ELISA</td>
</tr>
<tr>
<td>KIM-1</td>
<td>Urine</td>
<td>PT</td>
<td>Ectodomain shed into urine following injury; production upregulated during dedifferentiation in response to injury</td>
<td>Type 1 membrane glycoprotein; phosphatidyl serine receptor; confers phagocytic properties to tubular epithelia</td>
<td>ATN&lt;sup&gt;b&lt;/sup&gt;; CPB&lt;sup&gt;b&lt;/sup&gt;; transplant&lt;sup&gt;b&lt;/sup&gt;; mixed (consult)&lt;sup&gt;b&lt;/sup&gt;; nephrotoxin&lt;sup&gt;b&lt;/sup&gt;; contrast&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Cystatin C</td>
<td>Urine/serum</td>
<td>Nucleated cells</td>
<td>Serum: accumulates as filtration decreases; rationale derived from CKD studies urine: impaired PT metabolism</td>
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<td>CPB&lt;sup&gt;b&lt;/sup&gt;; ICU&lt;sup&gt;b&lt;/sup&gt;; contrast&lt;sup&gt;b&lt;/sup&gt;; obstruction&lt;sup&gt;b&lt;/sup&gt;; nephrotoxin&lt;sup&gt;b&lt;/sup&gt;; hemorrhagic fever&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Nephelometry; turbidimetry; ELISA</td>
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<td>IL-18</td>
<td>Urine/serum</td>
<td>PT; macrophages; fibroblasts; dendritic cells; intestinal epithelia; adrenal cortex</td>
<td>Upregulated in ischemic injury and released into urine</td>
<td>Inflammation; immunomodulatory</td>
<td>ATN&lt;sup&gt;b&lt;/sup&gt;; CPB&lt;sup&gt;b&lt;/sup&gt;; ARDS&lt;sup&gt;b&lt;/sup&gt;; ICU&lt;sup&gt;b&lt;/sup&gt;; contrast&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>NAG</td>
<td>Urine</td>
<td>PT</td>
<td>Lysosomal enzyme in PT, high molecular weight &gt;130 kD not filtered</td>
<td>Glucosidase</td>
<td>ICU&lt;sup&gt;b&lt;/sup&gt;; CPB&lt;sup&gt;b&lt;/sup&gt;; nephrotoxin&lt;sup&gt;b&lt;/sup&gt;; mixed (consult)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Colorimetric enzymatic assay</td>
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<tr>
<td>L-FABP</td>
<td>Urine</td>
<td>PT; liver; small intestine</td>
<td>Translocation from cytosol to tubular lumen during ischemic injury</td>
<td>Cytoplasmic protein involved in fatty acid trafficking; may scavenge reactive lipids</td>
<td>CPB&lt;sup&gt;b&lt;/sup&gt;; transplant&lt;sup&gt;b&lt;/sup&gt;; AAA repair&lt;sup&gt;b&lt;/sup&gt;; ICU&lt;sup&gt;b&lt;/sup&gt;; nephrotoxin&lt;sup&gt;b&lt;/sup&gt;; sepsis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ELISA</td>
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<tr>
<td>a/π GST</td>
<td>Urine</td>
<td>α-PT; π-DT</td>
<td>Cytoplasmic enzymes</td>
<td>Glutathione transferase conjugation of electrophilic substances</td>
<td>CPB&lt;sup&gt;b&lt;/sup&gt;; ICU&lt;sup&gt;b&lt;/sup&gt;; contrast&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ELISA</td>
</tr>
<tr>
<td>NHE-3</td>
<td>Urine</td>
<td>PT/TALH</td>
<td>Most abundant sodium transporter in PT; decrease in sodium resorption during course of AKI suggested loss in urine</td>
<td>Sodium transport</td>
<td>ICU&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Western blot</td>
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<tr>
<td>IL-6</td>
<td>Plasma</td>
<td>Macrophages; lymphocytes; smooth muscle</td>
<td>Increased production and impaired clearance associated with AKI development and prognosis</td>
<td>Inflammation, immunomodulatory</td>
<td>ICU&lt;sup&gt;b&lt;/sup&gt;; sepsis&lt;sup&gt;b&lt;/sup&gt;; transplant&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ELISA; Luminex (microsphere)</td>
</tr>
<tr>
<td>Netrin-1</td>
<td>Urine</td>
<td>PT blood vessels, lung, pancreas, mammary gland</td>
<td>Increased expression early after ischemia-reperfusion and nephrotoxin injury</td>
<td>Axonal guidance, developmental factor, renal function unclear</td>
<td>CPB&lt;sup&gt;b&lt;/sup&gt;; mixed (consult)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Western blot</td>
</tr>
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ICU, intensive care unit; ER, emergency room; CPB, cardiopulmonary bypass; NT, nephrotoxins; PT, proximal tubule; DT, distal tubule; TALH, thick ascending loop of Henle; ELISA, enzyme-linked immunosorbent assay.

<sup>a</sup>Pediatric.

<sup>b</sup>Adult.
renal azotemia, rhabdomyolysis, medications, or decreased production).

In the absence of a potentially more informative standard such as tissue examination, which is neither feasible nor ethical in most human studies of AKI, the use of creatinine as a comparator is not unreasonable. However, the resulting temptation has become to dismiss studies as positive or negative based solely on agreement with this imperfect standard. In this paradigm, a good performer may reflect the same intrinsic limitations as serum creatinine whereas a poor performer may be summarily dismissed as having little value despite potentially providing information not previously captured. The potential peril of relying exclusively on this approach has been illustrated by recent animal studies demonstrating markedly superior performance of KIM-1 and L-FABP for detecting confirmed pathologic injury from ischemia or nephrotoxins compared with serum creatinine. Clinical data suggesting the possibility that several candidate markers may be more sensitive than creatinine to kidney injury have also emerged.

Similar challenges would likely have faced if the validation of cardiac biomarkers (troponin and CK-MB) for myocardial injury in patients with chest pain were relegated to a single functional standard such as a 25% change in ejection fraction (Figure 2). Given similar sensitivity and specificity restrictions of creatinine and its attendant risk for misclassifying actual injury status, caution must be taken before new biomarkers are accepted or dismissed based upon this comparison alone. It follows that an important area remaining to be addressed in current analyses is a rigorous testing of the hypothesis that candidate markers are indeed providing novel information. One potential avenue worth exploring is the hypothesis that candidate markers are indeed providing novel information.

Figure 1. Phases of clinical biomarker evaluation and AKI studies. Adapted from the American Heart Association Scientific Statement Criteria for Evaluating Novel Markers of Cardiovascular Risk. $n$ = approximate number of human studies published with classification in multiple phases allowed. Early phases include both proof of concept studies (phase 1) demonstrating differences in biomarker levels between patients with and without the outcome of interest (i.e., AKI) and prospective studies (phase 2) to determine the association between levels and the risk of future hard outcomes such as dialysis requirement, graft survival, and mortality. Subsequent phases consider aspects of clinical incorporation including determining the incremental predictive value of a candidate marker beyond established risk predictors (phase 3) and if biomarker use changes therapy for at-risk patients, improves outcomes, and is cost-effective (phases 4 to 6). References are listed in Supplemental Table 2.

Figure 2. Potential limitations with using a functional gold standard as a surrogate for injury. Comparison of limitations using serum creatinine as a gold standard with example of validation of myocardial injury biomarkers made against a similar functional standard (change in ejection fraction). In the latter, an absence of a loss of LV function would be insufficient to rule out ongoing myocardial damage, especially if early or mild. Similarly, decrements in ejection fraction would not necessarily denote injury as other potential factors responsible (e.g., increased afterload, medications, and volume status).
Figure 3. Closer examination of patients according to biomarker and creatinine data agreement. In patients where biomarker and creatinine data agree (gray panels), the relationship between biomarker levels and the risk of additional outcomes should be confirmed. Patients in whom biomarker and creatinine data disagree (red panels) should also be closely examined. Increased risk for worsening/future AKI or adverse events in patients whom biomarker levels suggest injury where creatinine does not may further support biomarker usefulness (bottom left). Similarly, evidence of rapid reversibility or nonrenal causes of creatinine elevation where biomarker levels do not may also be informative (upper right). Supplementing this evaluation with tools to assess for evidence of tubular dysfunction including concentrating or diluting ability (FeH2O, FeUrea), acidosis, electrolyte complications, sediment, volume overload, and novel imaging techniques may help further characterize what new markers may be telling us.

More Effective Use of Serum Creatinine

As finding creative methods to expand AKI phenotyping beyond creatinine will undoubtedly be critical to advancing biomarker development, one immediate question is whether the current use of creatinine in biomarker studies is being optimized? While RIFLE/AKIN consensus definitions have facilitated identifying patterns of AKI across populations in epidemiologic studies, it may be that overreliance of a one size fits all approach to capturing AKI in biomarker validation studies carries the risk of extending the misclassification already inherent in its use, particularly with more mild injury definitions. For example, given the nonlinear relationship between GFR and serum creatinine, commonly used changes (0.3 to 0.5 mg/dl) may be less specific as an injury standard in those patients with underlying CKD, the population reported to be at highest risk for AKI. In a recent prospective study of 426 adults undergoing CPB, differences in early NGAL production between AKI and non-AKI patients were only observed in those with a baseline eGFR ≥60 ml/min. In patients whose baseline eGFR <60 ml/min, it was not clear how much the lack of difference in NGAL production was attributable to a native production or clearance deficit versus the effects of ascertaining bias (high false-positive rate) in capturing AKI using a 0.3 mg/dl change in this group. Yet in a recent study in emergency room patients, NGAL production in patients with CKD appeared sufficient to distinguish between established AKI and non-AKI status. Similarly, creatinine kinetic modeling has demonstrated the rate of creatinine elevation may be delayed in CKD relative to non-CKD patients suggesting that the interval for injury detection (usually 24 to 48 h in most studies) in this population may also warrant reconsideration. Finally, while recognizing the usefulness of cutoffs in clinical practice, the dichotomization of serum creatinine may be resulting in further loss of information including the tendency to lump patients with different patterns of creatinine and/or biomarker expression on either side of a given threshold. The latter is of particular concern given the limited sample sizes in most current AKI biomarker studies to date. Relying exclusively on such cutoffs may also preclude examination of potentially important aspects of AKI such as injury “trajectory”. Examples of the latter include recently proposed metrics of serum creatinine change to more effectively detail rate, degree, and duration of injury including the average change in creatinine above baseline (AVC) and the AVC relative to baseline (RAVC). Ultimately, if a future goal is to retain, rather than replace serum creatinine as one of several markers used to fully characterize AKI, then understanding the full relationship between these markers and creatinine will only be realized if the latter is more thoroughly explored.

Phase 2: Beyond Creatinine

As mentioned previously, extending the scope of focus toward less ambiguous outcomes, including mortality, delayed graft function, and dialysis is a critical component of ongoing validation of AKI biomarkers. Recalling the previous comparison to myocardial injury, it is worth noting that the clinical adoption of troponin derived largely from its ability to predict harder outcomes such as mortality in both biomarker positive, CK-MB elevated patients as well as biomarker negative, without CK-MB elevation patients. Furthermore, as most AKI occurs as a precipitant of spontaneous illness where the feasibility of early detection in practice may be hampered both by a lack of baseline creatinine data or renal-specific symptoms to prompt biomarker measurement, the ability to provide prognostic information in patients meeting minimal criteria of injury may be where the principal value in AKI biomarkers lies. Despite the large number of Phase 2 studies listed in Figure 1, most have been single-center and lack the number of events to sufficiently examine these types of outcomes in detail. For example, in a recent meta-analysis by Haase et al., 19 studies examining the diagnostic and prognostic utility of NGAL, the most-studied AKI marker to date, found the number of patients that required short-term dialysis or died as an outcome ranged from 4 to 22 and 3 to 52, respectively. This wobble underscores the need to establish or leverage existing multi-center cohorts where bio-
specimen collection and AKI data may already be captured. In addition to expanding statistical power to allow for interpretation and assessment of marker utility beyond established predictors, such efforts would also yield unique and important opportunities for cross-validation. Toward that end, it should be noted that several multi-center prospective studies in CPB and intensive care unit settings are ongoing or nearing completion and primed to compare the early diagnostic and prognostic utility of AKI biomarker panels.

BIOMARKER PROPERTIES

As data from several markers suggest performance variations depending on the clinical venue studied, questions arise regarding how biomarker expression within each setting may influence performance. While upregulated in renal tubular epithelia during isolated ischemia-reperfusion or nephrotoxic injury, markers such as NGAL, IL-18, and L-FABP, for example, are also produced in other cell types during acute illness.99–101 Because different threshold values provide optimal discrimination in various settings, it is not clear whether this reflects different severities of injury or the effects of systemic production.97 Such observations may help explain why detection of AKI during discrete ischemia-reperfusion injury such as CPB or kidney transplantation20,43,55,56 appears more robust than in critically ill children or adults with systemic illness.59,62,63 Additionally, studies in this latter group with IL-18 and NGAL suggest that predicting the presence or progression of injury may improve when septic patients are excluded.42,71 In addition to examining the relationships between urine and plasma concentrations or the fractional excretion of a given marker, other methods in development may help determine the renal specificity of a given urine biomarker. For example, both nucleic acid material and proteins have recently been isolated from urinary exosomes.102,103 Quantitative analyses also find upregulated expression of mRNA encoding KIM-1 or NGAL in renal tissue after ischemia-reperfusion, calcineurin toxicity, or interstitial fibrosis and tubular atrophy.104,105 Similar evidence of genomic expression of specific markers in the urine of patients with AKI may hold promise for confirming the renal origin of markers that are also produced systemically.106

In addition to elucidating organ-specific expression of urine biomarkers, continued investigation of the pattern of biomarker expression among different settings will be required to determine their optimal application. Numerous early detection studies in the CPB literature suggest that maximum discrimination of several markers among patients developing or not developing AKI occurs within a window of a few hours.20,43,46,107 Although the use of marker combinations with different rates of rise after an insult may help broaden the detection window, it can be argued that sustained expression may be a requisite feature for usefulness in settings where the onset is more difficult to anticipate, as recently demonstrated in a clinical trial of critically ill adults with AKI.108 Furthermore, extension of preclinical studies detailing the temporal pattern of expression of candidate markers beyond discrete ischemia-reperfusion or nephrotoxic injury to other models of injury such as sepsis will also be imperative to better inform study settings where injury is often multifactorial.109 Finally, study of expression patterns in specific at-risk populations, including the elderly or those with underlying CKD,87 will be needed as early data also suggest unique biomarker expression patterns in these groups of patients.88

Similar to studies of other complex acute diseases such as acute respiratory distress syndrome,110 a single biomarker is unlikely to be informative enough to account for the complexity of pathways, etiologies, and sites involved in the generation of AKI. Consequently, incorporation of several markers into a diagnostic or prognostic panel will likely be required to profile the nature of the insult and its effects. However, as multi-marker panels in AKI are developed, mindfulness of lessons learned from similar previous attempts in other diseases is worthwhile. For example, as two given markers may share similar biologic pathways, the incremental value of adding a marker that is highly correlated to another may be marginal if it is measuring information captured already. Thus, a rational approach to selecting markers for inclusion in panels should comprise efforts not only to match candidate markers to the type of anticipated injury, for example, S3 segment proximal tubular markers for ischemic AKI, but also to identify uncorrelated or orthogonal markers targeting distinct pathophysiological mechanisms.111,112 Such an approach might couple, for example, knowledge gained through preclinical studies comparing biomarker expression in different models of injury with statistical approaches that quantify the degree of colinearity between markers. An example of the former includes a unique partnership recently formed between biotechnical, pharmaceutical, and regulatory agencies to facilitate the qualification of AKI biomarkers for safety in drug development potentially targeting multiple anatomic sites of injury.113

ASCERTAINING CLINICAL UTILITY IN PHASE 3 AND BEYOND

An important aspect of the utility of newly discovered markers is determining to what extent a candidate marker provides incremental prognostic information relative to established risk markers.114 Does, for example, measurement of a new biomarker in patients with early AKI add to the ability to predict need for renal replacement therapy above and beyond demographic information and known indicators of illness severity? Conventional approaches to answer this question include examining the degree of independent statistical association by multivariable regression analyses, discrimination, and calibration. Similar to phase 2 studies, few phase 3 studies listed in Figure 1 have had sufficient events to examine this question in detail. Discrimination, the most commonly used tool in diagnostic AKI biomarker studies, em-
ploys traditional characteristics of test performance including sensitivity, specificity, and predictive values to generate summary characteristics, most notably the AUC-ROC curve.\textsuperscript{115,116}

The AUC-ROC curve measures the probability of a marker to correctly rank order any two randomly selected patients, one experiencing the outcome of interest, such as AKI, and one who does not. Given its versatility, the AUC-ROC curve is often extended to the prediction of future events. Yet observations, primarily from the cardiovascular literature, suggest that change in the AUC-ROC may be a relatively insensitive metric to assess the incremental predictive ability of a marker relative to established predictors.\textsuperscript{117} In addition to the stochastic nature of future events, it has been estimated that extremely robust associations between biomarker levels and the outcome of interest, with minimal overlap in the distribution, are required to substantially improve the AUC.\textsuperscript{118,119}

The accuracy or calibration of a model is another important measure that attempts to quantify the risk of a given patient for a particular outcome and reflects how well the observed risk of an event for individuals within a cohort matches the predicted risk based on a set of covariates. While important in helping determine an individual patient’s level of risk for AKI or its attendant outcomes, calibration also frequently depends on the choice of risk categories.

Because of these limitations, newer methods to assess how a marker improves classification across a wide spectrum of risk have been developed. These include the integrated discrimination improvement index, which determines the extent to which a marker correctly increases or decreases the degree of risk and its extension, and the net reclassification index, which determines the proportion of patients reclassified appropriately to another predefined threshold of risk that might, for example, alter treatment decisions.\textsuperscript{120} It is notable that few studies in AKI thus far have attempted to integrate some of these latter techniques within their performance evaluation.\textsuperscript{56,59}

Ultimately, clinical usefulness of a set of markers will be determined not only by accuracy and reliability in prediction but also by whether that information alters therapy or improves clinical outcomes. Examples include safety monitoring during the administration of new and established nephrotoxins, aiding the identification of patients at highest risk for requiring renal replacement therapy, monitoring responses to an intervention, predicting long-term sequelae of AKI such as CKD, and correctly identifying patients at sufficient risk to warrant exposure to novel AKI interventions. While perhaps not yet sufficiently validated for the latter, inclusion of both biomarker positive and negative patients in this setting may potentially provide a useful venue for continued validation as illustrated in a hypothetical trial design (Figure 4).\textsuperscript{121}

CONCLUSION

After decades of being deemed a mere byproduct of severe illness, AKI has gained recognition as a serious contributor to short- and long-term morbidity and mortality. Newly discovered tissue-specific markers have the potential to transform the characterization and phenotyping of this increasingly common disease beyond our continued reliance on blending retrospective changes in estimated GFR with clinical impression. Critical steps to avoid becoming lost in translation include broadening of the interface between preclinical and clinical data to help inform the choice, timing, and setting for testing putative markers, creative approaches on how to best incorporate, rather than replace, serum creatinine, and the careful application of novel statistical methods to ascertain clinical value. Coupled with concerted efforts to establish larger cohorts with sufficient event rates for adequate validation of harder end points, increased rigor in the examination of these markers represents a long overdue opportunity to improve outcomes in this disease.

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