Application of new acute kidney injury biomarkers in human randomized controlled trials

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Abstract

The use of novel biomarkers of acute kidney injury (AKI) in clinical trials may help evaluate treatments for AKI. Here we explore potential applications of biomarkers in simulated clinical trials of AKI using data from the TRIBE-AKI multicenter, prospective cohort study of patients undergoing cardiac surgery. First, in a hypothetical trial of an effective therapy at the time of acute tubular necrosis to prevent kidney injury progression, use of an indirect kidney injury marker such as creatinine compared to a new direct biomarker of kidney injury reduces the proportion of true acute tubular necrosis cases enrolled. The result is a lower observed relative risk reduction with the therapy, and lower statistical power to detect a therapy effect at a given sample size. Second, the addition of AKI biomarkers (interleukin-18 and NGAL) to clinical risk factors as eligibility criteria for trial enrollment in early AKI has the potential to increase the proportion of patients who will experience AKI progression and reduce trial cost. Third, we examine AKI biomarkers as outcome measures for the purposes of identifying therapies that warrant further testing in larger, multicenter, multi-country trials. In the hypothetical trial of lower cardiopulmonary bypass time to reduce the risk of postoperative AKI, the sample size required to detect a reduction in AKI is lower if new biomarkers are used to define AKI rather than serum creatinine. Thus, incorporation of new biomarkers of AKI has the potential to increase statistical power, decrease the sample size, and lower the cost of AKI trials.
AKI is a research priority; it has many causes (surgery, infection, medication), affects 10% of hospitalized patients, is difficult to manage, is associated with poor outcomes, and is very costly. Although many therapies have ameliorated AKI in preclinical animal studies, few therapies have proven beneficial in human testing. There are 2 major challenges that have hampered AKI drug development.

The first challenge has been the inability to adequately phenotype this syndrome. The diversity of AKI subtypes (hemodynamic, intrarenal, postrenal) and etiologies (septic, ischemic, nephrotoxic), coupled with the lack of accurate and rapid diagnostic tests that can distinguish between the different forms of AKI, has led to challenges in the diagnosis, classification, and prognosis of patients with the disease. In other words, it has proven difficult to enroll the “right patient” at the “right time” in AKI clinical trials.

The second challenge has been the lack of validated outcome measures for AKI clinical trials. Currently, outcome measures used in early-phase clinical trials rely on disease classification systems such as AKIN, RIFLE, KDIGO, and their derivatives. These systems are primarily based on changes in the serum creatinine concentration, an indirect measure of kidney injury. Its concentration is dependent on several clinical variables, such as hydration, muscle metabolism, and medication effects. Standardization generally assists with reporting of results; however, it is unclear whether a treatment’s effect on AKI, as defined by these disease classification systems, will predict its effect on clinical outcomes.

A solution to both of these major challenges may lie in the development of more accurate AKI biomarkers.

KIDNEY INJURY BIOMARKERS: VALUE IN DRUG DEVELOPMENT

As in clinical practice, biomarkers are important tools in drug development. A biomarker is a measurable indicator of normal biologic processes, pathologic processes, or biologic responses to a therapeutic intervention. Biomarkers can be used in several ways to facilitate the conduct of AKI clinical trials. At enrollment they can be used as an “enrichment strategy” to (i) preferentially enroll patients with a certain type of AKI (diagnostic biomarkers), (ii) identify patients more likely to progress to a higher stage of AKI (prognostic biomarkers), and (iii) identify a subpopulation of patients most likely to respond to a particular intervention (predictive biomarkers). They can also be used as outcome measures to monitor response to therapy (pharmacodynamic biomarkers), whether it be beneficial or harmful to a patient (Figure 1). The terminology used in this article reflects the terminology currently adopted by the US Food and Drug Administration (FDA) to describe the biomarker uses within the context of drug development. As noted by the FDA, these methods of biomarker use are not mutually exclusive; for example, in diabetic kidney disease trials, urine albumin is used as a prognostic biomarker to preferentially enroll...
patients at higher risk of reaching the trial’s end point, and as a pharmacodynamic biomarker to support proof-of-concept studies and aid in dose selection. Novel biomarkers used in isolation may not perform as well as a composite biomarker score in combination with existing clinical information and traditional tests.

To examine the potential utility of new AKI biomarkers in drug development trials, we analyzed data from the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) cohort, a multicenter, prospective cohort of patients undergoing cardiac surgery. We evaluated biomarkers in various roles (diagnostic, prognostic, predictive, and pharmacodynamic) in hypothetical and simulated AKI trials, and demonstrate their impact on detectable relative risk, required sample size, and trial cost.

RESULTS

Diagnostic biomarker of true kidney injury

Current AKI diagnostic criteria rely on the serum creatinine concentration, which is a filtration marker and indirectly measures kidney injury. Use of serum creatinine in prior clinical trials may have led to the enrollment of heterogeneous patient populations, where only a subpopulation had an AKI subtype likely to respond to the intervention being tested. In this regard, new diagnostic AKI biomarkers, which are released upon injury to specific renal tubular segments, may more accurately reflect a diagnosis of acute tubular necrosis (ATN). Interleukin 18 (IL-18), released from the proximal tubule upon injury, has been shown to differentiate ATN from other forms of kidney disease. Biomarkers such as neutrophil gelatinase–associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and liver-type fatty acid–binding protein (L-FABP), as well as traditional biomarkers such as urine microalbumin and fractional excretion of sodium, also help diagnose ATN. Traditional and novel biomarker combinations, including filtration and injury biomarkers, could be used in clinical trials to enroll patients with a form of AKI most likely to respond to an intervention. Selection of these patients for enrollment would be expected to reduce the sample size needed to detect a therapeutic effect if it in truth exists.

For example, a hypothetical drug that treats ATN may not be effective in treating other conditions that raise the serum creatinine concentration. Such conditions include hemodynamic causes such as volume depletion, hepatorenal and cardiorenal syndromes, glomerulonephritis, acute interstitial nephritis, and urinary tract obstruction. If a clinical trial uses elevation of serum creatinine as the enrollment criterion, it will invariably lead to enrollment of patients with and without ATN. Table 1 models the results of a trial of a hypothetical therapy for the early treatment of AKI that reduces the risk of AKI progression by 30% in patients with true ATN, but has no effect in patients without ATN. Depending on the non-ATN patient proportion in the enrolled trial sample, this could result in a substantial loss of statistical power (Figure 2a) and require a higher sample size to reliably detect the true treatment effect if it exists (Figure 2b).
Prognostic biomarkers

AKI prognostic biomarkers can help identify a patient subpopulation at high risk of developing severe AKI or other poor clinical outcomes. Enrolling patients at greater risk of reaching these study end points could lead to a substantial reduction in the required sample size and duration of follow-up for trials (recognizing there is additional effort to find patients who are eligible for trial participation). In other renal settings such as diabetic kidney disease and polycystic kidney disease, prognostic biomarkers (albuminuria, reduced glomerular filtration rate [GFR], total kidney volume) have been used in drug development as inclusion criteria to enrich clinical trials. A number of observational studies have evaluated prognostic biomarkers (novel, traditional, risk models) of AKI in various clinical settings to identify high-risk patients; these studies indicate that measuring biomarkers at or before the time of AKI can improve our ability to detect patients at high risk for progressing to more severe AKI or a longer duration of AKI. Therefore, these prognostic biomarkers may enrich AKI trials. An example of this approach and its impact on the trial cost is presented in Table 2.

To explore the effects of an enrichment strategy for enrolling patients at high risk for AKI soon after surgery, in the TRIBE-AKI cohort we utilized a combination of a known renal insult (cardiopulmonary bypass [CPB] time >120 minutes) and novel biomarkers (plasma NGAL and urine IL-18) obtained within 6 hours after the surgery (but before development of clinical AKI). We calculated the rate of developing severe AKI with various enrichment strategies, and calculated the sample size needed to detect a relative risk of 0.7 of a given intervention with 80% power and 5% alpha. We used the following assumptions: The cost of screening was $500 based on the cost of blood and urine testing and research staff effort in the extra screening step. The cost per patient enrolled in a trial was variable, and depended on the intervention cost, follow-up duration, and follow-up laboratory investigation cost. A recent cost analysis of phase 3 trials in obesity, cardiovascular disease, and diabetes showed that $45,000 to $50,000 was spent per patient enrolled. We also did the cost analysis for a less expensive intervention by assuming $10,000 cost per patient, which may be realistic for a therapy in AKI that does not require many years of follow-up. By using a combination of known insult (CPB time >120 minutes) and injury markers (IL-18 or NGAL), we demonstrate that trial cost can be decreased by 29% to 64% (Table 2). The main drawback of using a highly selective strategy, as evident from these tables, is the high screen failure rate and the need to screen a large number of AKI patients (which would require additional clinical sites and investigators). Second, since this approach uses biomarkers obtained around the time of injury (cardiac surgery), it will not be applicable to therapies that need to be administered before the injury.

Predictive biomarkers

Predictive biomarkers identify the patients most likely to respond to a particular treatment based on the candidate drug’s mechanism of action. They are a powerful enrichment strategy and reduce trial size and cost by identifying a subgroup of patients who have higher likelihood of responding to intervention and thus demonstrate a larger effect size. Predictive biomarkers have been used with success in other diseases to enhance clinical trials and provide personalized treatments. A classic example of this strategy is the drug Herceptin.
(trastuzumab). In clinical trials, it was found that HER-2–overexpressing breast cancer patients had 5-month survival with Herceptin as compared to 2-month survival in patients overall.\textsuperscript{22,23} Similarly, using a patient’s molecular signature in AKI as an inclusion criterion could aid in the evaluation of novel therapeutics and personalized AKI treatments. For example, a drug acting on the apoptosis pathway via the NLRP-3 inflammasome complex would be more effective in patients in whom this AKI pathway is active. A biomarker, such as urine IL-18, that is released upon activation of the NLRP-3 inflammasome pathway could be a predictive biomarker to select patients for enrollment in a trial of such a drug.

While both prognostic and predictive biomarkers can be used as enrichment strategy, there is an important distinction in their application. Prognostic biomarkers identify a subgroup of patients at higher risk of an event (e.g., need for dialysis, progression to a higher stage of AKI, mortality) and enrich the cohort enrolled in a trial for the desired event. Prognostic biomarkers can improve the absolute risk reduction achieved by an intervention by increasing the overall event rate but do not change the relative risk reduction. The biomarker used in prognostication may not be causally related to AKI progression, or be a target for therapy. Predictive biomarkers, on the other hand, identify a subgroup of patients who are most likely to respond to a particular intervention and in theory improve both the observed relative and absolute risk reduction with a therapy. A predictive biomarker is often a therapeutic target or causally related to the outcome.

**Pharmacodynamic biomarkers**

Pharmacodynamic biomarkers assess whether a biological response, favorable or unfavorable, has occurred in a patient receiving a therapeutic intervention. As end points, they assist in detecting efficacy or toxicity of the intervention and quantify the extent of this response, thereby also allowing selection of optimal dose of the intervention.

**Efficacy**—Pharmacodynamic biomarkers indicating efficacy are valuable in early proof-of-concept trials. Biomarkers that respond rapidly to treatment could facilitate early decision making and shorten often lengthy proof-of-concept studies. Biomarkers examining clinical treatment effectiveness (i.e., improved survival or reduced disease progression) are being tested and validated in various forms of chronic kidney disease. For example, a reduction in proteinuria in patients with diabetic kidney disease is associated with a decreased progression from chronic kidney disease to end-stage renal disease.\textsuperscript{24,25} Furthermore, efficacy biomarkers can be incorporated in adaptive trial designs, where change in biomarkers on follow-up samples can be used to modify sample size or inclusion criteria during the course of the trial, further streamlining the clinical trial process. Pharmacodynamic biomarkers of efficacy may have potential of being accepted as surrogate outcomes if the effect of a specific treatment on biomarkers correlates strongly and consistently with meaningful clinical outcomes. While cross-sectional and short-term prospective studies have identified a number of biomarkers associated with AKI, there is a lack of evidence from longitudinal and interventional studies that validate the utility of any biomarker for monitoring disease activity or clinical response.
**Safety**—Pharmacodynamic biomarkers that monitor toxicity or harm hold potential to serve as safety end points in clinical trials, particularly in phase 1 and 2 trials for dose selection. In fact, such application to monitor safety in drug development is quite prevalent. For example, serum creatinine is routinely monitored in the drug development process of nonrenal drugs to detect kidney toxicity during dose selection. However, serum creatinine lacks sensitivity and specificity for detecting true kidney injury and is not an ideal biomarker for safety. Biomarkers with higher sensitivity for kidney injury may be preferable as better markers of kidney damage. A panel of new urine biomarkers recently received FDA qualification for monitoring renal toxicity in preclinical studies.

**Application of efficacy biomarkers in clinical trials**—To demonstrate the potential application and benefit of a pharmacodynamic biomarker in AKI clinical trials, we use TRIBE-AKI data and simulate trials of 2 interventions, which in the literature have been shown to reduce the risk of AKI. In the first example, we use CPB time as an intervention to show that a smaller sample size would be needed with biomarkers to demonstrate the beneficial effect of shorter CPB time on kidney injury (Table 3, Supplementary Table S1 online). In the second example, we show that the beneficial effect of statins on AKI is evident if biomarkers are used as outcomes, whereas creatinine-based outcomes are not statistically significant (Figure 3).

**Shorter CPB time trial**—Longer CPB time is a known risk factor for AKI after cardiac surgery. In the TRIBE-AKI adult cohort, we simulated a trial by using short CPB time as an intervention to reduce AKI incidence. We selected patients who underwent surgery with a short CPB time (<80 minutes, n = 141) and matched them with patients who underwent surgery with a long CPB time (>120 minutes, n = 141) using a propensity score (see Materials and Methods for matching algorithm). Baseline characteristics of the patients were similar in the 2 matched groups (Supplementary Table S1). We evaluated the incidence of AKI defined by the fifth quintile of first postoperative biomarker following short versus long CPB time (Table 3). We calculated the sample size necessary to detect a statistically significant difference in various biomarker-defined AKI end points between the long and short CPB time groups. As shown in Table 3, the sample size needed to detect a difference in outcomes was substantially smaller when AKI was defined by first urinary NGAL, L-FABP, and IL-18 as a pharmacodynamic end point (n = 95, 119, and 204, respectively) as compared to AKI defined by a doubling of serum creatinine or dialysis as the end point (n = 1945). Thus, sensitive pharmacodynamic biomarkers could reduce the sample size of early-phase AKI trials by reclassifying a significant proportion of patients in whom AKI is absent when assessed with serum creatinine values (Table 3).

**Statin trial**—Statins are hypothesized to be renoprotective due to their antioxidant and anti-inflammatory properties, and animal studies have shown that statins are renoprotective when administered before an ischemia–reperfusion injury. Prior studies evaluating the role of statins in AKI (using a creatinine-based definition) showed negative results. However, several observational studies and meta-analysis of clinical trials strongly suggest a protective role of statins in AKI. In concordance with these observations, recent work has
demonstrated that statins are correlated with decreased AKI biomarkers after cardiac surgery.\textsuperscript{31}

We selected patients from the TRIBE-AKI cohort who were already on statins (n = 625) and divided them into 2 groups based on whether statins were continued (n = 131) or held (n = 494) on the day of surgery. As the study protocol did not specify whether statins were to be continued or held, and no guidelines exist on perioperative statin management, the decision to continue or hold statins was at the discretion of individual care providers. We evaluated the effect of statins on AKI as assessed by serum creatinine and kidney injury biomarkers. A total of 25 of 625 patients (4\%) experienced AKI as defined by serum creatinine doubling or receipt of dialysis, and statin continuation had no effect on serum creatinine–based AKI (adjusted relative risk 1.09; 95\% confidence interval 0.44–2.70; statin held as referent group); statin continuation did show a statistically significant decrease in AKI defined by fifth quintile of peak biomarker levels (Figure 3; adjusted relative risk [95\% confidence interval]: urine IL-18 0.34 [0.18–0.62], P < 0.0001; urine NGAL 0.41 [0.22–0.76], P = 0.001; KIM-1 0.37 [0.2–0.67], P < 0.0001).\textsuperscript{31} The first postoperative biomarkers were also statistically significant for urine IL-18 and urine NGAL. These results suggest that variability and insensitive quality of serum creatinine may be insufficient to detect statin’s renoprotective effect after cardiac surgery, and kidney injury biomarkers may serve as better pharmacodynamic biomarkers of early AKI clinical trials.

DISCUSSION

The pharmaceutical industry, recognizing the unmet need for AKI therapeutics, has made a considerable effort in advancing drug candidates for clinical use. There are at least 10 therapeutics currently in AKI clinical trials. However, the path to new therapies has been littered with many failed clinical trials, the majority of them failing in early phases after promising results in preclinical experiments. The question remains, however, whether the previously failed drug candidates had the wrong target or suboptimal trial designs. An important step in conducting effective trials is reducing the heterogeneity of AKI patients enrolled in clinical trials by selecting patients most likely to respond to intervention, and it seems unlikely that traditional biomarkers alone will be sufficient for appropriate phenotyping of AKI. Novel biomarkers can play a role in differentiating ATN from other forms of AKI, selecting patients at appropriate stage and severity of AKI, or selecting patients likely to progress to higher stages of AKI. Biomarkers can also measure therapy effects, both favorable and unfavorable, in early-phase clinical trials. Adequately validated biomarkers could reduce the sample size, improve the statistical power, and save promising therapies from false-negative results. Thus, biomarkers can help address some of the challenges associated with developing AKI treatments (Table 4).

Many new AKI biomarkers are being evaluated in common clinical settings of AKI, and some biomarkers are potentially ready for incorporation in clinical trials. For example, several urine biomarkers of tubular injury diagnose ATN. The ability of urine biomarkers to increase 24 to 48 hours before serum creatinine during an episode of AKI could also be leveraged in clinical trials for early drug administration. In early AKI treatment trials, the
prognostic ability of kidney injury biomarkers to predict progressive AKI or long-term mortality is promising in the perioperative setting.

The level of evidence needed before biomarkers can be qualified for use in clinical trials or practice depends on their desired context of use. Biomarker validation is an iterative process requiring multiple studies to identify, characterize, and confirm consistency of any observed associations. In order to fully capture the multiple AKI clinical manifestations and apply them reliably to clinical trial design, new strategies that rely on a panel of several novel biomarkers will be necessary, and a collection of multiple biomarkers could lead to the generation of a testable composite AKI biomarker score. It is important for translational research scientists to coordinate efforts with regulatory agencies and the pharmaceutical industry to select and develop a validated biomarker toolbox for use in AKI trials. Biomarker development and drug development can be an integrative process with biomarker validation occurring concurrently during the conduct of clinical trials. Pharmaceutical companies and investigators should commit to the evaluation of biomarkers within clinical trials and to information sharing to improve our understanding of AKI. Biomarker development is a long-term investment but one that the AKI community has recognized and is embracing as critical in the path toward a successful AKI therapy.

Limitations of biomarkers

Biomarkers have certain important limitations that need to be considered when applied in clinical trials. Use of biomarkers to select patients for enrollment in a trial leads to a higher screen failure rate and requires a larger number of patients to be screened for enrollment. Moreover, enrollment based on biomarkers may limit the generalizability of results. Predictive biomarkers are usually therapeutic targets or causally related to outcomes and can only be tested in the setting of a therapy when the molecular action is well known. Finally, biomarkers are important as outcome measures in early-phase trials to facilitate decisions around advancing the therapeutic to large-scale multicenter trials. However, there is a lack of information from longitudinal and interventional studies that validate the utility of any biomarker from early-phase studies for predicting patient-centered outcomes in larger-scale trials.

METHODS

The data for the various simulations demonstrated in this paper were obtained from the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) study. The TRIBE-AKI study is a prospective cohort of 1219 adults with at least 1 risk factor for AKI who underwent cardiac surgery (coronary artery bypass graft or valve surgery). Participants were enrolled at 6 academic medical centers in North America from July 2007 through December 2009. Full study details have been previously described. High risk for AKI was defined by the presence of 1 or more of the following: emergency surgery, preoperative serum creatinine >2 mg/dl, ejection fraction <35% or grade 3 or 4 left ventricular dysfunction, age >70 years, diabetes mellitus, concomitant coronary artery bypass graft and valve surgery, or repeat revascularization surgery. For biomarker measurement, we collected plasma and urine specimens preoperatively and daily until at
least postoperative day 3 in all patients. Informed consent was obtained from all patients or their proxy decision makers. The study was approved by the institutional review board at each participating institution. AKI biomarker measurement methods have been previously described.\textsuperscript{18,33} We recorded serum creatinine values obtained in routine clinical care for every patient throughout the hospital stay. We calculated preoperative estimated GFR using the Chronic Kidney Disease Epidemiology Collaboration equation.\textsuperscript{34} We defined AKI clinically by a change in serum creatinine of $\geq 50\%$ or $\geq 0.3$ mg/dl from preoperative to peak postoperative value and severe AKI as AKI with doubling of serum creatinine or requiring dialysis. Fifty-two patients (4.3\%) developed severe AKI.\textsuperscript{35} We collected preoperative characteristics, operative details, and postoperative complications using definitions of the Society of Thoracic Surgeons.\textsuperscript{32}

Simulated intervention trials

To demonstrate the potential application and benefit of pharmacodynamic biomarkers in AKI clinical trials, we used TRIBE-AKI data and simulated trials of 2 interventions: (i) CPB time (<80 minutes or >120 minutes); (ii) statin management (continued or held on day of surgery). To simulate the effect of randomization in an actual clinical trial of low versus high CPB time, we matched treatment-and control-arm patients (1:1) on the basis of baseline characteristics (age, gender, preoperative CKD [estimated GFR $\leq 60$ ml/min per 1.73 m$^2$], site) and propensity score (based on baseline characteristics of diabetes, hypertension, congestive heart failure, contrast exposure, preoperative medication exposure [angiotensin-converting enzyme inhibitors, $\beta$-blockers, and statins]).

We examined clinical AKI event rates defined by serum creatinine in each arm of the simulated trial, and AKI defined by elevated biomarker levels using the fifth quintile of biomarker values measured in the entire TRIBE-AKI cohort. The cut points for the fifth quintile of the first postoperative biomarkers were: IL-18, 60 pg/ml; NGAL, 102 ng/ml; KIM-1, 1.19 ng/ml; L-FABP, 175 ng/ml. We were able to ascertain AKI outcome measurements on all individuals in the simulated trial, and there was no loss to follow-up. Unadjusted relative risks were calculated based on the observed event rates in the hypothetical treatment and control arms.

To examine the difference in the AKI definitions (elevated serum creatinine vs. elevated biomarker), we examined reclassification tables. The reclassification percentage was calculated in the control arm and was defined as the proportion of individuals with improved reclassification when defining AKI by elevated biomarker levels compared to clinical AKI defined by serum creatinine elevation. Improvement in reclassification occurred in 1 of 2 scenarios: (i) there was no clinical AKI by serum creatinine, but AKI by elevated biomarker levels, or (ii) there was clinical AKI by serum creatinine but no AKI by elevated biomarker levels.

Sample size calculations

Sample size calculations for a specified risk reduction were based on 2-sided test with 0.05 alpha and 80\% power (unless otherwise specified). We assumed 2 equal-sized groups.
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References


Diagnostic biomarkers can help enroll a subpopulation of patients with a given type of acute kidney injury (AKI), such as acute tubular necrosis (ATN). Prognostic biomarkers can be used to enroll a subpopulation who are at greater risk of poor clinical outcomes including AKI progression. Pharmacodynamic biomarkers can be used to assess AKI treatment effects that may be either beneficial or harmful (i.e., efficacy and safety outcomes). These biomarkers may also help define who is responding well to the intervention (to determine whether the dose or agent should be changed). Clinically Meaningful Outcomes: therapies showing promise with favorable pharmacodynamic biomarker response (possibly in the vanguard phase of trial rollout) can be tested in longer-duration trials, or trials with larger sample sizes, to assess therapy effects on clinically meaningful outcomes, such as receipt of acute dialysis, progression to chronic kidney disease or end-stage renal disease, mortality, length of hospital stay, hospital readmissions, patient symptoms, and quality of life.
Figure 2. Effect of enrolling different proportions of patients with ATN in a clinical trial with an intervention that has relative risk reduction of 30% in patients with ATN, and no effect in patients without ATN.

(a) Statistical power increases with increasing proportion of patients with acute tubular necrosis (ATN) among AKI participants. (b) Required sample size decreases with increasing proportion of patients with ATN among AKI participants. The treatment and control groups are assumed to be of equal size. Alpha = 0.05. For a, the event rate in patients with ATN in the treatment group is 14%, and in the control group it is 20%. The event rate in patients without ATN is 5%. For b, power is set at 80%. Event rates in patients with ATN are noted in the figure. The event rate in patients without ATN is 5%.
Figure 3. Effect of continuation of statins (vs. holding of statins) on various biomarkers of AKI after cardiac surgery in the TRIBE-AKI study

Severe AKI: Doubling of serum creatinine or requiring dialysis. AKI based on biomarkers was defined as the fifth quintile (urine IL-18 >201 pg/ml, urine NGAL >156 ng/ml, urine KIM-1 >15.8 ng/ml, plasma NGAL >307 ng/ml, urine L-FABP >199 ng/ml, urine albumin >121.6 mg/l). Peak biomarker levels were defined as the highest biomarker value in the first 3 postoperative days. We adjusted the analysis for age, gender, race, diabetes, type of operation (coronary artery bypass graft [CABG] and valve vs. CABG or valve), preoperative estimated GFR with CKD-EPI equation (<60, ≥60), congestive heart failure, cardiac catheterization, preoperative urine albumin-to-creatinine ratio (<10, 10–30, >30), preoperative angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, preoperative diuretic (yes/no), preoperative calcium channel blocker (yes/no). AKI, acute kidney injury; CI, confidence interval; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid–binding protein; NGAL, neutrophil gelatinase–associated lipocalin; pNGAL, plasma NGAL; RR, relative risk; uNGAL, urinary NGAL. P < 0.0001 for IL-18 and KIM-1, P = 0.001 for uNGAL, P = 0.2 for pNGAL, P = 0.6 for L-FABP. The 95% CIs are shown on a log_{10} scale. Figure adapted using data from Molnar et al.\textsuperscript{31} See article for full details.
Table 1

Example of early treatment trial in acute kidney injury

<table>
<thead>
<tr>
<th>AKI etiology</th>
<th>Intervention event rate</th>
<th>Control event rate</th>
<th>Observed relative risk</th>
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<tr>
<td>100% ATN + 0% non-ATN</td>
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</tr>
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Impact of changing the proportion of enrolled patients with true acute tubular necrosis (ATN) in a trial where a therapy reduces the relative risk (RR) of progressive kidney injury by 30% in patients with ATN, but has no effect on outcomes in the absence of ATN. We assume that 95% of non-ATN cases will respond to standard of care, and the acute kidney injury (AKI) will not progress. With these assumptions, the table shows the effect on observed RR with increasing proportion of non-ATN cases. Examples of calculation for 60% ATN + 40% non-ATN: Intervention rate: 60% (14%) + 40% (5%) = 10.4%. Control rate: 60% (20%) + 40% (5%) = 14%. RR = 10.4%/14% = 0.74.

*Treatment administered at the time of clinical diagnosis of AKI by serum creatinine.*
Table 2

Cost reduction by using biomarkers as enrollment criteria to predict clinical acute kidney injury

<table>
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<tr>
<th>Enrollment criteria</th>
<th>Sample size details</th>
<th>Trial screening</th>
<th>Total trial cost (screening + trial)(^a)</th>
<th>% Cost reduction(^f)</th>
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<td>AKI event rate control arm</td>
<td>Required sample size (RR 0.7)</td>
<td>Screening failure rate</td>
<td>Screening cost (in dollars)</td>
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</table>

AKI, acute kidney injury; AKI event, doubling of serum creatinine or requiring dialysis; pNGAL, plasma neutrophil gelatinase–associated lipocalin; RR, relative risk; uIL-18, urinary interleukin-18.

\(^a\)Expressed in millions.

\(^b\)TRIBE-AKI cohort (high risk for AKI based on clinical risk factors; see Materials and Methods for details).

\(^c\)Cardiopulmonary bypass time >120 minutes.

\(^d\)Cost estimated assuming lower cost of $10,000 per patient, which may be more appropriate for a short-duration AKI therapeutic trial.

\(^e\)Cost estimated based on $45,000 per patient. This cost is estimated based on recently reported pharmaceutical-sponsored trials in cardiology and endocrinology (from Roy\(^2\)).

\(^f\)Percentage reduction in cost is calculated using trial with clinical risk factors as reference.
Table 3
Sample size for hypothetical cardiopulmonary bypass time trial using various biomarkers as pharmacodynamic end points

<table>
<thead>
<tr>
<th>Clinical AKI defined by serum creatinine</th>
<th>Reclassification % in control arm, AKI by biomarker versus clinical AKI&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event rate in control arm (CPB time &gt; 120 min)</td>
<td>Minimal detectable relative risk</td>
</tr>
<tr>
<td>AKI &gt; 50% or dialysis</td>
<td>26%</td>
</tr>
<tr>
<td>AKI &gt; 100% or dialysis</td>
<td>7%</td>
</tr>
<tr>
<td>AKIN stage 1 or higher</td>
<td>49%</td>
</tr>
<tr>
<td>AKI defined by elevated biomarker levels&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>26%</td>
</tr>
<tr>
<td>uNGAL</td>
<td>33%</td>
</tr>
<tr>
<td>KIM-1</td>
<td>24%</td>
</tr>
<tr>
<td>L-FABP</td>
<td>28%</td>
</tr>
<tr>
<td>pNGAL</td>
<td>26%</td>
</tr>
<tr>
<td>Urine albumin</td>
<td>18%</td>
</tr>
<tr>
<td>IL-18 &amp; uNGAL</td>
<td>21%</td>
</tr>
</tbody>
</table>

AKI, acute kidney injury; CPB, cardiopulmonary bypass; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid–binding protein; pNGAL, plasma neutrophil gelatinase-associated lipocalin; uNGAL, urine NGAL.

<sup>a</sup>AKI based on biomarkers was defined as first postoperative biomarker value in the fifth quintile. Cut points for fifth quintile: IL-18, 60 pg/ml; uNGAL, 102 ng/ml; KIM-1, 1.19 ng/ml; L-FABP, 175 ng/ml; pNGAL, 293 ng/ml. Sample size calculation based on 2-group test with equal numbers in each group. Two-sided alpha of 5%, statistical power 0.8 for the reported relative risk.

<sup>b</sup>Reclassification percentage was calculated in the control arm as the percentage of individuals with improved reclassification when defining AKI by elevated biomarker levels compared to clinical AKI defined by elevation in serum creatinine. Improvements in reclassification occurred in the following 2 circumstances: (i) there was no clinical AKI by serum creatinine, but AKI by elevated biomarker levels, or (ii) there was clinical AKI but no AKI by biomarker elevation (see shaded cells in Supplementary Table S2).
Table 4

Impact of biomarkers on future acute kidney injury trials

<table>
<thead>
<tr>
<th>Problems with current trial design</th>
<th>Role of biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Misclassification: inaccurate definition of “true AKI” or ATN</td>
<td><em>Diagnostic biomarkers:</em> Use of urine biomarkers can lead to more accurate diagnosis of AKI and recruitment of a more homogenous patient population</td>
</tr>
<tr>
<td>2. Enrollment of large proportion of low-risk patients who do not reach progression end points</td>
<td><em>Prognostic biomarkers:</em> Identification of high-risk patients likely to reach trial end points</td>
</tr>
<tr>
<td>3. All patients enrolled in trials are given similar therapy</td>
<td><em>Predictive biomarkers:</em> Patients most likely to respond to a particular therapy are identified</td>
</tr>
<tr>
<td>4. Current outcomes of efficacy and progression take months or years to develop; potentially beneficial therapies are terminated before reaching end points or harmful therapies are continued without recognition of harm</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pharmacodynamic biomarkers:</em> Trials can be continued to harder end points or terminated early based on their effect on biomarkers. Such biomarkers may serve to monitor safety or efficacy.</td>
</tr>
</tbody>
</table>

AKI, acute kidney injury; ATN, acute tubular necrosis.

*Enrichment strategy: Prognostic and predictive biomarkers used as enrollment criteria can be used as “enrichment strategy.”*