

The Role of Biomarkers in Cardiac Surgery-associated Acute Kidney Injury

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Introduction

Cardiac surgery associated-acute kidney injury (AKI) is common, affecting up to 40 % of heart surgery patients (depending on the definition used) [1]. Five to ten percent of these patients will require new renal replacement therapy, and this carries a high price in terms of morbidity, mortality and resource utilization [2]. Despite decades of successful animal research, there have been remarkably few positive human clinical trials of cardiac surgery associated-AKI prevention and treatment, and no treatment has been confirmed effective in large, multicenter randomized clinical trials [3]. Much of the problem has been attributed to delayed diagnosis – serum creatinine does not rise until 48 hours after surgery – such that by the time the diagnosis is made, it is too late to intervene. In prevention trials, lack of a clear consensus definition of AKI has hampered endpoint adjudication such that it still remains unclear which therapies may be effective, in which patient subtypes they should be used, and what is the correct time to begin and end them. The advent of a new generation of biomarkers coming hot on the heels of a consensus definition system for AKI [4] means that we may be poised to make significant advances in this area. This chapter reviews the place of existing and novel biomarkers in the diagnosis, risk stratification and prognosis of cardiac surgery associated-AKI.

What are the Characteristics of an Ideal Biomarker of Cardiac Surgery-associated-AKI?

The ideal biomarker should provide quick, reliable, and inexpensive measurement of a biological process that is inextricably linked to cardiac surgery associated-AKI. It should be readily quantifiable in accessible clinical samples (e.g., plasma or urine), with levels greatly increased specifically in cardiac surgery associated-AKI. There should be minimal to no overlap in biomarker levels between cases and control subjects, nor should levels be subject to wide variation in the general heart surgery population. Biomarker levels should correlate with the total burden of disease, but be unaffected by unrelated conditions and associated comorbid factors, and should correlate closely with the established parameters of disease that are known to influence quality of life and survival. They should vary rapidly in response to specific treatments, and large deviations of the biomarker from the reference values in the control population should have predictive power for disease severity and prognosis. Such a biomarker does not exist, nor is it likely to, and it is more probable that pan-

els of biomarkers assessing different aspects of renal function and damage will be most useful in the management of patients with cardiac surgery associated-AKI [5].

Diagnostic Performance: How Good is a Test?

Before attempting to understand how good any particular biomarker is for the diagnosis of cardiac surgery associated-AKI, it is important to understand the terms commonly used to describe the measures of accuracy of any diagnostic test, such that meaningful interpretation of the results of studies evaluating novel biomarkers may be made.

Diagnostic tests may return values that are categorical (has AKI/does not have AKI), that are ordinal (has stage 1, 2 or 3 AKI) or that are continuous (quantitative measurement of functional defect). In general, categorical tests may be assessed in terms of sensitivity, specificity, positive predictive value, negative value, and receiver operating characteristic (ROC) curve analysis. For diagnostic purposes, continuous tests are usually dichotomized into yes/no categories and sometimes also into bins of severity to permit simpler interpretation. Although this makes them easier to understand, in general continuous variables are more powerful descriptors of physiological aberration and thus many biomarker discovery studies have used this approach instead of straightforward categorical descriptions. Below we outline what is meant by the commonly used descriptors of diagnostic test performance.

- Sensitivity (true positive rate): This is the probability that a test will make a diagnosis of AKI in a patient who really does have AKI. It is the true positive rate, and is given by the fraction of all AKI patients who have a positive test.
- Specificity (true negative rate): This is the probability that a test will make a diagnosis of no AKI in a patient who really does not have AKI. It is the true negative rate, and is given by the fraction of patients without AKI who have a negative test.
- Positive predictive value (precision rate): This is the proportion of patients with positive test results who are correctly diagnosed. It is given by the fraction of positive test results that actually have AKI. It is dependent on the prevalence of the disease however, increasing in direct proportion with disease prevalence.
- Negative predictive value: This is the probability that a patient with a negative test does not have AKI. It is given by the fraction of patients with negative test results that indeed truly do not have AKI. The various operating characteristics of diagnostic tests are summarized in [Table 1](#).

One of the problems commonly facing diagnostic tests is that the operating characteristics (sensitivity and specificity) may change across the potential range of cut-off

Table 1. Features of a diagnostic test

	Acute kidney injury	No acute kidney injury	Utility
Positive test	True positive	False positive (Type 1 error)	Positive predictive value
Negative test	False negative (Type 2 error)	True negative	Negative predictive value
Test characteristic	Sensitivity	Specificity	

values for a 'positive' diagnosis. A test that is 100 % sensitive but 10 % specific will not retain much clinical utility, particularly if the consequences of an erroneous diagnosis are not trivial. In practice, the operating characteristics of a test across different cut off values may be assessed using a ROC curve [6]. This curve plots the true positive rate (sensitivity) against the false positive rate (1-specificity), and the area under the curve (AUC) provides a single estimate of how 'good' the test is for the diagnosis of a disease. Generally, values of 0.8 and higher are accepted as being clinically useful. A perfect classifier would lead to a curve where the elbow of the curve lay on the upper left corner of the plot, and would have an AUC of 1.0.

What Biomarkers are Needed?

Given the limitations of using serum creatinine and urine output for detecting AKI, there is a need for better biomarkers that can more reliably help refine the diagnosis of AKI [7]. Potentially, these novel biomarkers may allow subcategorization of this disease in a manner analogous to the way hematological malignancies have been subcategorized, now that information about their molecular subtypes is available. It is probable that cardiac surgery associated-AKI is not a single disease, rather a panoply of illnesses that appear similar from a functional deficit perspective, but that are mediated by different pathological processes. This may also explain why we have failed to translate therapies that are effective in (single mechanism) animal models of AKI into the human domain.

If a panel of biomarkers could be developed such that the diagnosis of AKI could be made soon after the injury (i.e., within hours), then the window of opportunity for effective therapies may be advanced earlier in the disease process, and potentially even during the initiation or propagation phase. In addition, an earlier and more reliable diagnosis of AKI would facilitate better risk stratification. Examples of outcomes that could be predicted might include AKI patients who subsequently require renal replacement therapy, duration of AKI, development of subsequent chronic kidney disease, and all cause mortality. Analogous to the cardiology composite of MACE (Major Adverse Cardiac Events), these outcomes may collectively be considered as a composite renal variable known as Major Adverse Kidney Events (MAKE).

What Biomarkers are Currently Available and How Good are They?

The biomarkers currently available to us for the diagnosis of cardiac surgery associated-AKI are unsatisfactory [8]. This is somewhat surprising since AKI is diagnosed exclusively on changes in laboratory parameters (serum creatinine, blood urea nitrogen). Once renal dysfunction develops it can be defined in a number of ways. The variables traditionally used to characterize postoperative renal dysfunction include peak postoperative serum creatinine, absolute and/or percentage change in serum creatinine, postoperative estimated creatinine clearance, absolute and/or percentage change in creatinine clearance, and need for renal replacement therapy [5]. All have previously been shown to be predictive of adverse postoperative outcomes.

Until the advent of cystatin C (see below), there had been no new tests of renal function in widespread use for more than 50 years. Increasingly, laboratories are beginning to offer cystatin C as a test of renal function, in the belief that it is a better

indicator of renal filtration function than estimates of serum creatinine. Whether this is actually true or not is unknown.

The most important reason to find a replacement for serum creatinine for the diagnosis and prognosis of AKI in general, and cardiac surgery associated-AKI in particular, is that there is a delay of at least 48 hours after an insult before the serum creatinine rises. This means we cannot currently intervene until it is too late; indeed, it is like providing coronary revascularization two days after a myocardial infarction. Novel tests of actual kidney damage (urinary neutrophil gelatinase-associated lipocalin [NGAL], kidney injury molecule [KIM]-1) may allow earlier diagnosis of tubular damage, rather than declining function, which in turn may allow much earlier interventions to be tested.

What Biomarkers are Under Development?

Prompted by these potential benefits of an improved biomarker for detecting AKI, several new biomarkers have been identified while some previously identified ones have been more intensely studied [9]. Although there have been over 20 unique biomarkers of AKI identified or under investigation, most of the current interest has focused on a handful of promising biomarkers: NGAL, cystatin C, interleukin (IL)-18, and KIM-1 [10].

Neutrophil Gelatinase-associated Lipocalin

NGAL is an immunological protein that is covalently bound to gelatinase from neutrophils and expressed at low levels by various human tissues including the kidneys. In the setting of cardiac surgery, NGAL has been demonstrated to be a highly sensitive and specific biomarker of postoperative AKI. Its gene is one of the earliest and the most upregulated in the kidney after ischemic injury. In one study of 71 children undergoing cardiopulmonary bypass (CPB), the incidence of AKI was found to be 28 %. Both urine and serum NGAL increased 2 hours after CPB and were found to be the most powerful independent predictors of AKI in this population. Two additional studies have demonstrated that both urine NGAL (at 2 hours) and plasma NGAL (at 12 hours) strongly correlate with mortality in children [11, 12]. Similar results have been observed in the adult cardiac surgical population [13]. In a study of 81 adult cardiac patients, 20 % of the patients developed postoperative AKI: NGAL was higher in patients with AKI at 1 hour, 3 hours, and 18 hours post-CPB when compared with their non-AKI counterparts.

A more recent study found that the use of aprotinin versus epsilon amino-caproic acid in patients undergoing cardiac surgery resulted in a twofold incidence of AKI in the aprotinin group. Urinary NGAL was significantly higher at both 0 and 3 hours post-CPB in patients receiving aprotinin [14]. Whether urinary NGAL may turn out to be a better predictor of cardiac surgery-associated-AKI than plasma NGAL remains to be demonstrated conclusively. One recent study found this to be the case [15], suggesting that plasma NGAL elevations may be more indicative of a global inflammatory insult induced by the CPB machine rather than a specific kidney insult. If this is the case, then the combination of plasma and urinary NGAL data may allow a more temporal approach to AKI diagnosis than has been possible previously.

Interleukin-18

IL-18, a pro-inflammatory cytokine that belongs to the IL-1 superfamily, has been shown to be both a mediator and biomarker of ischemic AKI. In a study of 55 children following CPB, urinary IL-18 was detectable at 4–6 hours, peaked at 12 hours, and remained elevated for over 48 hours [16]. Further multivariate analysis suggested that urine IL-18 may also be a marker of AKI severity. Similar results have been also been observed in non-cardiac populations with AKI [17–19].

Kidney Injury Molecule-1

KIM-1 is an immunoglobulin superfamily transmembrane protein normally present at low levels in proximal renal tubular cells that dramatically increases in expression following acute ischemic or nephrotoxic insult [20]. Recent findings in cell cultures also demonstrate that it transforms renal epithelial cells into ‘semiprofessional’ phagocytes that may assist with clearance of the apoptotic and necrotic cells that result from AKI [21]. Several studies in non-cardiac patients have demonstrated that KIM-1 is a very sensitive indicator of AKI [20–22]. However, fewer studies exist specifically in the cardiac surgery literature. In a cohort study of 103 adult patients undergoing CPB, KIM-1 levels increased significantly at both 2 hours and 24 hours postoperatively in patients with AKI [22].

Cystatin C

Cystatin C is a protein that is produced by all nucleated cells [23]. It has been suggested that cystatin C is an ideal molecule for measuring glomerular filtration rate (GFR) because it is freely filtered by the glomerulus, completely reabsorbed by the proximal convoluted tubules, and is not secreted [23, 24]. Unlike creatinine, it is not affected by age, gender, sex, or body mass [24]. There have only been a few studies to date that have explored cystatin C as a biomarker for AKI post-cardiac surgery [15]. In this prospective study, cystatin C and NGAL were measured in serum and urine samples of 72 adults who underwent cardiac surgery. Within the first 6 hours, serum values for cystatin C and NGAL were not predictive of AKI while urinary values were elevated. These findings suggest that urinary biomarkers may be superior to serum values for early detection of AKI.

Limitations of New Biomarkers

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Despite the promise of earlier detection of AKI, and with greater sensitivity, each biomarker has its limitations. NGAL may be influenced by pre-existing renal disease as well as infections [25]. KIM-1 is more specific for ischemic and nephrotoxic AKI and may not be useful in detecting other types of renal injury [20]. IL-18 peaks later than many other leading biomarkers, and is more specific for ischemic AKI [16]. Finally, cystatin C is not specific for ischemic AKI and its serum level rises much later than NGAL, KIM-1, and IL-18. In addition to their individual limitations, a variety of statistical and methodologic issues must be appropriately considered when determining the overall diagnostic performance of biomarkers. First, odds ratios or relative risks alone are inadequate to discriminate between individuals who may or may not have AKI. Rather, the accuracy and validity of biomarkers are better

summarized using ROC curves as discussed earlier [8]. Next, errors in the reference standard may threaten the validity of biomarker studies, but may be addressed by using hard outcomes (e.g., renal replacement therapy-requiring AKI, death) that are not subject to the same potential for misclassification that is inherent in diagnosing AKI. Additionally, risk prediction in individual patients requires that the biomarker have strong discriminatory characteristics that include a large difference in risk between low versus high values of the biomarker. Finally, the diagnostic characteristics of biomarkers will need to be evaluated with a consistent gold standard at well-defined time points.

Although determining their individual test characteristics will represent a significant advance in this field, combinations of biomarkers in multi-marker panels may provide a leap forward with the ability to differentiate between various AKI phenotypes and detect ischemic AKI earlier with greater precision and prognostic capacity.

One further limitation of the use of urinary protein levels for detection of cardiac surgery-associated-AKI is the fact that antifibrinolytic agents such as aminocaproic acid and tranexamic acid are both known to induce a temporary proteinuria during their administration [26]. Although this phenomenon is temporary and self limiting, it is important to consider when interpreting urinary protein data in patients who have received these drugs.

How will New Biomarkers Change the Diagnosis and Treatment of AKI?

As mentioned above, novel biomarkers of acute kidney damage and acute deteriorations of renal function will allow earlier diagnosis of a defect, and potentially may allow us to refine the diagnostic approach to AKI by pointing to disease mechanisms. This in turn may allow more targeted application of salvage therapies, and even preventive therapies if there are common pathophysiological pathways involved [8].

Second, new biomarkers of prognosis are required in order that we may predict who will require renal replacement therapy and who will not [27]. If a person will ultimately require renal replacement therapy then there is little to be gained by delaying its initiation, since all that will generally occur is the development of fluid and electrolyte abnormalities that will take longer to correct once renal replacement therapy is finally begun. Biomarkers of disease progression, then, will also be important to identify.

Third, new biomarkers of outcome will greatly assist with individual patient prognostication. If it could be shown that certain patterns in relevant biomarkers are associated with consistent outcomes, then this will assist physicians as they make difficult decisions about ongoing treatment in advanced multiorgan disease. Acute renal failure is almost always a feature of multiple organ failure, and if it is known that permanent renal replacement therapy will be required because the kidneys have failed to a point where they will never recover then this information will be important to integrate into the overall clinical decision making process.

Conclusion

In summary, there has been a lack of new biomarkers for more than half a century. Accompanying the recent consensus on diagnosis of AKI (albeit one which uses serum creatinine which itself may one day be replaced) has been the discovery and

initial validation of new biomarkers of both renal function and kidney damage. This will hopefully allow more focused investigation of their role in disease diagnosis, prognosis and classification such that we do not have to face a further 30 years of failed translational research. Indeed, it may be the case that we should re-explore some therapies that have been consigned to the pharmacological trash can, in the light of new information about disease mechanisms in different clinical settings. Lastly, cardiac surgery associated-AKI represents a prime arena for such investigation, since we know precisely when the insult occurs, and are ideally poised to intervene.

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