Evaluation of 32 urine biomarkers to predict the progression of acute kidney injury after cardiac surgery

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Biomarkers for acute kidney injury (AKI) have been used to predict the progression of AKI, but a systematic comparison of the prognostic ability of each biomarker alone or in combination has not been performed. In order to assess this, we measured the concentration of 32 candidate biomarkers in the urine of 95 patients with AKIN stage 1 after cardiac surgery. Urine markers were divided into eight groups based on the putative pathophysiological mechanism they reflect. We then compared the ability of the markers alone or in combination to predict the primary outcome of worsening AKI or death (23 patients) and the secondary outcome of AKIN stage 3 or death (13 patients). IL-18 was the best predictor of both outcomes (AUC of 0.74 and 0.89). L-FABP (AUC of 0.67 and 0.85), NGAL (AUC of 0.72 and 0.83), and KIM-1 (AUC of 0.73 and 0.81) were also good predictors. Correlation between most of the markers was generally related to their predictive ability, but KIM-1 had a relatively weak correlation with other markers. The combination of IL-18 and KIM-1 had a very good predictive value with an AUC of 0.93 to predict AKIN 3 or death. Thus, a combination of IL-18 and KIM-1 would result in improved identification of high-risk patients for enrollment in clinical trials.

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Acute kidney injury (AKI) is increasing in frequency[1] and is associated with a high incidence of adverse outcomes.[2] Identification of biomarkers that diagnose or predict the magnitude of AKI after cardiac surgery has been a goal of investigators for over a decade. The most well-studied biomarkers are those that reflect an inflammatory process in AKI, such as interleukin (IL)-18,[3] and biomarkers that have increased tubular cell synthesis following renal injury, such as neutrophil gelatinase–associated lipocalin (NGAL)[4] and kidney injury molecule-1 (KIM-1).[5] Recently, there has been an increased interest in the use of combinations of biomarkers to predict the development of AKI.[6] Combinations could account for differing time courses of biomarker release,[7] or they could reflect different pathophysiological mechanisms. In a recent study, the area under the curve (AUC) values to predict AKI after cardiac surgery were 0.65 for KIM-1, 0.61 for N-acetyl-β-D-glucosaminidase, and 0.67 for NGAL. The combination of the three markers had an AUC of 0.78 to predict the development of AKI.[8] Biomarkers could also be added to clinical variables. Addition of L-fatty acid–binding protein and N-acetyl-β-D-glucosaminidase to a clinical model improved the ability to predict the development of AKI after cardiac surgery from an AUC value of 0.79–0.86.[9]

Recently, the identification of biomarkers that predict the outcomes of patients with established AKI rather than the development has been highlighted. Predictive biomarkers could be used to select patients at higher risk of adverse outcomes. Identification of patients with existing AKI who will develop worsening kidney disease would enable more timely interventions. The recent KDIGO (Kidney Disease: Improving Global Outcome) clinical practice guidelines for AKI suggest that consideration of intensive care unit admission, renal replacement therapy, and adjustments in drug dosing be made for patients with more severe AKI.[10]

Biomarkers have been used to predict worsening AKI among patients with AKI, but these studies have attempted to predict any change in AKI (defined as worsening of Acute Kidney Injury Network (AKIN) stage) rather than development of severe AKI defined as stage 3 or death.[11,12] The single study that has attempted to predict the development of severe AKI at the time of AKI diagnosis demonstrated that urine NGAL had an AUC value of 0.78, although only nine patients progressed to severe AKI.[13] Interventions could be made earlier if patients at high risk of worsening AKI could be identified. Predictive biomarkers could also be used to guide enrollment in clinical trials, allowing for selection of patients most likely to benefit from intervention. Individual biomarkers have not been robust predictors of worsening AKI. Although studies have used combinations of biomarkers to predict the development of AKI,[6,9,14] fewer have used biomarker combinations to predict the worsening of AKI.[12] We measured 32 candidate biomarkers in patients with stage 1 AKI after cardiac surgery to determine the ability of the biomarkers alone or in combination to predict worsening AKI.

RESULTS

Urine samples and clinical data were collected from 95 subjects who had AKIN stage 1 AKI at the time of urine collection after cardiac surgery. Seventy-three of these patients achieved a maximum AKIN stage of 1, of whom 1 died; 12 patients had a maximum stage of 2, with 2 deaths; and 10 patients had a maximum stage of 3, with 6 deaths. Twenty-three patients met the combined end point of AKI progression (reaching AKIN stage 2 or 3) or death within 30 days of the urine sample collection. There was no difference between the outcome groups based on gender, race, comorbidities, type of surgery, baseline serum creatinine, creatinine at collection, and time to collection from the time of surgery (Table 1). We measured the concentration of 32 urine analytes in order to determine the ability of each biomarker to predict the combined outcome of AKI progression or death. Seven of the biomarkers had at least 19 ($\geqslant$20%) samples for which the biomarker was 'out of range low' (OOR$<$), a situation in which the fluorescent signal falls below the lower asymptote of the fitted dose–response curve, thereby precluding concentration estimation. Primary analysis was performed using the AUC of the receiver operating characteristic curve using a leave-one-out cross-validation approach. Initial analysis (Supplementary Tables S1 and S2 online) showed that adjustment for urine creatinine improved the ability to predict the outcome for most of the biomarkers, and thus adjusted values are reported. In contrast to most of the markers, NGAL had slightly higher predictive values for both end points without adjustment. To provide an initial framework for characterization of the biomarkers, we divided the biomarkers into mechanistic groups. Table 2 shows the predictive characteristics for each of the 32 urine analytes broken down by biomarker functional category. We ranked biomarkers by their mean squared error (MSE)—lower MSE indicates better fit—and evaluated predictive performance using AUC. The highest AUC value of 0.74 was seen for IL-18 (Figure 1) and renin. KIM-1 had an AUC of 0.73 and VEGF, IL-6, and NGAL all had AUC of 0.72. For comparison, percentage of change in serum creatinine at the time of collection and Cleveland Clinic scores had AUC values of 0.76 and 0.64, respectively.

We next compared the ability of the biomarkers to predict the outcome of development of severe AKI (defined as AKIN stage 3) or death within 30 days. Baseline demographic and clinical characteristics were similar between groups (Supplementary Table S3 online). Change in serum creatinine and Cleveland Clinic score showed only marginal improvements in prediction, but the predictive ability of many of the biomarkers was markedly improved (Table 3). IL-18 was the best predictor with an AUC of 0.89 and the smallest MSE. Figure 1 shows the receiver operating characteristic curves for prediction of both outcomes and boxplots for the values of creatinine-adjusted IL-18 for each of the eventual outcomes. These data demonstrate that the currently available biomarkers are better predictors of severe AKI than they are for the outcome of any degree of worsening in AKI and that IL-18 is an excellent predictor of severe AKI or death.

To determine the relationship of individual biomarkers with each other, we performed two analyses. First, we performed an unsupervised cluster analysis to determine which biomarkers were similar to each other (Figure 2). We found many similarities to our *a priori* grouping of biomarkers, but we also found interesting differences. Many of the proteins that we had proposed were filtered plasma proteins that are not reabsorbed in the tubule because of tubular dysfunction were grouped together (albumin, alpha-1 antitrypsin, cystatin C, beta-2 microglobulin, and retinol-binding protein). Similarly, many of the proteins we described as inflammatory proteins were also grouped together. However, some of the proteins that we thought would be similar to each other were clustered differently. NGAL and KIM-1, which were placed in the injury response (up) group, were geographically distant from each other in the dendrogram. We also compared correlation coefficients for each of the biomarkers within the groups and with the best marker in each of the other groups (Supplementary Tables S4–11 online). Overall, the correlation within each group was stronger for biomarkers that had better predictive ability. A notable exception was KIM-1, which had a poor correlation with other markers in its group, as well as with markers in other groups (Supplementary Table S6 online). KIM-1 was a strong predictor of both outcomes (AUC = 0.73 and 0.81) but had a correlation coefficient of 0.20 with L-FABP and of 0.24 with IL-18, suggesting that the combination of KIM-1 with one of these other markers may be beneficial.

We determined the ability of combinations of biomarkers to predict the two outcomes, ranking groups of biomarkers according to MSE. The combination of IL-18 and percentage of change in serum creatinine (Table 4) had the lowest MSE