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Combination of biomarkers for diagnosis of acute kidney injury after cardiopulmonary bypass

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Abstract

Novel acute kidney injury (AKI) biomarkers offer promise of earlier diagnosis and risk stratification, but have yet to find widespread clinical application. We measured urinary α and π glutathione *S*-transferases (α-GST and π-GST), urinary ʟ-type fatty acid-binding protein (ʟ-FABP), urinary neutrophil gelatinase-associated lipocalin (NGAL), urinary hepcidin and serum cystatin c (CysC) before surgery, post-operatively and at 24 h after surgery in 93 high risk patient undergoing cardiopulmonary bypass (CPB) and assessed the ability of these biomarkers alone and in combination to predict RIFLE-R defined AKI in the first 5 post-operative days. Twenty-five patients developed AKI. π-GST (ROCAUC = 0.75), lower urine Hepcidin:Creatine ratio at 24 h (0.77), greater urine NGAL:Cr ratio post-op (0.73) and greater serum CysC at 24 h (0.72) best predicted AKI. Linear combinations with significant improvement in AUC were: Hepcidin:Cr 24 h + post-operative π-GST (AUC = 0.86, $p = 0.01$), Hepcidin:Cr 24 h + NGAL:Cr post-op (0.84, $p = 0.03$) and CysC 24 h + post-operative π-GST (0.83, $p = 0.03$), notably these significant biomarkers combinations all involved a tubular injury and a glomerular filtration biomarker. Despite statistical significance in receiver-operator characteristic (ROC) analysis, when assessed by ability to define patients to two groups at high and low risk of AKI, combinations failed to significantly improve classification of risk compared to the best single biomarkers. In an

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alternative approach using Classification and Regression Tree (CART) analysis a model involving NGAL:Cr measurement post-op followed by Hepcidin:Cr at 24 h was developed which identified high, intermediate and low risk groups for AKI. Regression tree analysis has the potential produce models with greater clinical utility than single combined scores.

Keywords

Acute kidney injury; biomarkers; cardiac bypass; glutathione *S*-transferase; hepcidin; liver fatty acid binding protein; neutrophil gelatinase associated lipocalin

Introduction

Acute kidney injury (AKI) is associated with increased morbidity and mortality[1] and is of particular clinical significance in the intensive care unit. Conventional diagnosis remains dependent on measurement of plasma creatinine.[2] However, plasma creatinine has many limitations, in particular, diagnosis is often delayed and imprecise in the setting of critical illness.[3,4] Delay in diagnosis of AKI may preclude early and effective intervention. A number of early markers of AKI have been identified by proteomic analysis of plasma and urine from patients who go on to develop AKI.[5,6] These biomarkers may accelerate AKI diagnosis and provide insights into its pathogenesis. However, while performance can be very good in homogeneous patient groups with predictable timing of renal injury, such as pediatric cardiopulmonary bypass – CPB,[7] diagnostic utility can be less good in a mixed populations of adults with chronic kidney disease,[8] or when renal insults vary in timing, nature and severity.[9] Furthermore, biomarkers of AKI are heterogeneous molecules. Some appear to be produced directly within the kidney as a result of tubular injury or stress (urinary neutrophil gelatinase-associated lipocalin – NGAL[10]); others may appear in the urine due to failure of proximal tubular uptake (urinary cystatin c[11]) while others again may simply be more precise markers of alteration in glomerular filtration rate (serum cystatin c – CysC[11]). Finally, some biomarkers of AKI may also be markers of systemic inflammation, complicating their interpretation; for instance the major source of plasma NGAL may be neutrophils in normal conditions and during systemic infection, but can be kidney derived during AKI.[12]

Thus, markers of tubular injury may specifically detect some patients with early AKI and relatively preserved GFR, while markers of GFR changes may detect hemodynamically mediated decreases in glomerular filtration rate in the absence of significant tubular injury. Logically, therefore, examination of multiple biomarkers may inform us not only of the likely occurrence of AKI, but its nature, extent and pathogenesis.

Accordingly, we examined the diagnostic utility of a panel of six biomarkers measured at baseline and at two post-operative time-points in a single-centre randomized pilot controlled trial conducted in higher-risk patients undergoing surgery involving CPB.[13] We hypothesized that some combinations of biomarkers would provide better diagnostic accuracy than either alone and that these combinations might involve biomarkers that reflect differing aspects and phases of the pathogenesis of AKI in this population.

Materials and methods

Patient population

Samples were obtained from 93 patients enrolled in the Cardiopulmonary bypass, Renal injury and Atorvastatin Trial (CREAT—Clinicaltrials.Gov Nct00910221), a single-centre randomized controlled trial of peri- and post-operative atorvastatin versus placebo for the prevention of AKI in higher risk patients undergoing CPB. This study was approved by Human Research Ethics Committee of the Austin Hospital. In this study, statin therapy did not influence the incidence of post-operative AKI or biomarkers of renal injury.[13,14] Renal function was monitored for 5 days post-operatively. Samples of serum and urine were obtained pre-operatively, immediately on return to ICU after surgery (mean time of 4.50 h; 3.58–9.13 h after CPB) and 24 h after surgery. Of the 100 patients randomized, five withdrew or had surgery rescheduled and in two patients, full sets of serum and urine were not collected for clinical reasons, so that 93 complete sets of urine samples were available for analysis. Demographic and clinical data were collected pre-operatively and over the first 24 h post-surgery. Serum creatinine was measured pre-operatively and daily for 5 days after surgery. AKI was defined using the creatinine criteria of the Risk, Injury, Failure, Loss, End-stage kidney disease (RIFLE) consensus definition of AKI.[15] As data on hourly urine output were not available for the complete study period, and in keeping with previous studies of CPB-associated AKI,[16,17] urine output definitions in the RIFLE criteria were not employed. Primary definition of AKI was the occurrence of RIFLE Class R or greater (a >50% rise in creatinine from baseline) in the five post-operative days. Secondary biomarker analysis for RIFLE-Injury or greater and a composite of need for renal replacement therapy or death was also conducted.

Biomarkers measurements

Samples for biomarker analysis were stored at −70 °C until the end of the study period and dispatched on dry ice for analysis. Co-investigators performing biomarker assays were blinded to patient details and AKI classification. We assessed four biomarkers of tubular injury: urinary α and π glutathione *S*-transferases (α-GST and π-GST),[17–19] urinary L-type fatty acid-binding protein (l-FABP)[20] and urinary NGAL,[5,21,22] and two biomarkers that potentially reflect changes in glomerular filtration: urinary hepcidin[23–25] and serum CysC.[11] We have reported on the utility of urinary hepcidin and NGAL for diagnosis of AKI in this study cohort previously.[13,14] α-GST and π-GST were measured by enzyme immunoassay (Argutus Medical, Ltd., Dublin, Ireland)[26] and expressed in ng/mL. For urinary L-FAB all samples were assayed using a sandwich ELISA[27] and reported in ng/mL. Urinary NGAL was measured by enzyme-linked immunosorbent assay[7] and reported in ng/mL. Urinary hepcidin was measured by competitive enzyme-linked immunoassay (C-ELISA) as previously described at Intrinsic LifeSciences, LLC (La Jolla, CA) and reported in ng/mL. Serum CysC was measured using nephelometric technology on a Beckman Image Analyzer (Beckman Coulter, Brea, CA) and reported in mg/L. Serum and urinary creatinine was measured using the modified Jaffe method standardized by isotope dilution mass spectroscopy. Urinary biomarkers were assessed both with and without normalization against urinary creatinine concentration to control for urinary dilution where recommended.

Statistical analysis

Receiver–operator characteristic (ROC) analysis, logistic regression analysis, Net Reclassification Index (NRI) calculation and decision tree analysis were performed using *R: A language and environment for statistical computing* (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org) utilizing the packages *pROC*,[28] *rpart* and *rms* (http://biostat.mc.vanderbilt.edu/rms).

Categorical data were reported as percentages, and compared using Fisher's exact test. Continuous data were reported as median with inter-quartile range (IQR) and compared using the Mann–Whitney *U* test. For comparisons, statistical significance was denoted by two sided p values of < 0.05, adjustment for multiple comparisons was performed using the Bonferroni correction.

The ability of biomarkers to predict AKI was assessed by plotting ROC curves and reported as area under the curve (AUC) with 95% confidence intervals[29] and p value for significance deviation from the null model AUC of 0.5. ROC curve optimal cut-off values for diagnosis, for curves with a statistically significant AUC, were defined as the point which maximized the Youden index, defined as: sensitivity + specificity—1.[30] Biomarkers that were associated significantly differed between No AKI and RIFLE R or greater AKI on univariate comparison after correction for multiple comparisons were considered in combination. When assessing combinations NGAL and Hepcidin were considered normalized to urinary creatinine. Combinations of biomarkers were screened by multiple logistic regression analysis and considered significant if the p value for the least significant biomarker reached significance level after correction for multiple comparisons. Predictive models for significant biomarker combinations were then derived from the optimal weighted linear combination of the two biomarker measurements (see Supplementary material), an approach that makes no assumptions about the distributions of sample data.[31] Assessment of the statistical difference between paired ROC curves for optimal combination of biomarkers and individual biomarkers was performed using DeLong's test for two correlated ROC curves[32] with a one-tail comparison of ROC curves. For significant biomarker combinations, the enhancement predictive ability after addition of a second biomarker was assessed by calculation of the NRI and Integrated Discrimination Improvement (IDI).[33,34] In addition, the ROC curve optimal cut-off for AKI was assessed against the cut-off of the best individual biomarker by calculation of the two-category NRI. Finally all individual biomarkers significantly predictive of AKI univariate analysis were considered in a classification and regression tree (CART) analysis for combined prediction of AKI. The CART method involves the segregation of different values of classification variables through a decision tree composed of progressive binary splits and has been applied to risk prediction in critical illness.[35] To avoid over-fitting, the tree was pruned to minimize the cross-validated error and risk of AKI was calculated for each of the terminal nodes in the CART to generate the risk stratification model.

Results

Patient characteristics

Of the patients in this study, 25 of 93 developed AKI as defined by RIFLE ≥ R (27%). Fourteen patients developed RIFLE ≥ I AKI (15%) and 10 RIFLE F (9.3%). Five patients received renal replacement therapy (RRT) and two died in hospital (one received RRT and died). Of the 25 patients with RIFLE-R or greater AKI, 16 had achieved RIFLE-R by creatinine criteria (50% rise in serum creatinine) by post-operative day 1. However, only 8 of 14 patients going on to develop RIFLE I or F and only 5 of 10 developing RIFLE F satisfied creatinine criteria for RIFLE-R on post-operative day 1. Patient characteristics are summarized in Table 1.

Individual biomarkers

Individual biomarkers performance is provided in Table 2. No biomarker was significantly associated with AKI when measured pre-operatively (Table 2). All urinary biomarkers rose significantly after CPB both in patients who developed AKI and those that did not. Immediately after surgery, urinary NGAL, urinary NGAL:Cr ratio, urinary π-GST, urinary L-FAB and serum CysC were associated with statistically significant ROC-AUC's (Table 2). The best ROC-AUC was for π-GST at 0.75. At the 24 h time-point, urinary L-FAB and π-GST were not associated with AKI; however higher urinary NGAL, NGAL:Cr ratio and serum CysC remained associated with AKI, while *lower* urinary hepcidin and hepcidin:creatinine ratios were significantly associated with AKI. Again the best AUC was 0.77 for the hepcidin: Cr ratio at 24 h.

Most biomarkers showed lesser ability to predict RIFLE I or F (Supplemental Table 1). However, urinary hepcidin at 24 h and serum CysC at 24 h were associated with increased ability to predict more severe AKI (AUC 0.84 and 0.80, respectively). Due to the small number of patients with RIFLE I this category was not considered separately. When considering patients who required RRT and/or died in hospital (6/93), urinary hepcidin at 24 h, urinary NGAL at 24 h and serum CysC at 24 h best predicted these outcomes during ICU admission with AUCs of 0.8–0.91, however, the number of outcomes was small and confidence intervals wide (Supplemental Table 2).

Biomarker combinations

Post-operative urinary NGAL:Cr ratio and urinary π-GST, and 24 h urinary hepcidin:Cr ratio and serum CysC significantly differed between AKI and no-AKI after correction for multiple comparison (Table 2) and were considered in combination. Three combinations were significant: urinary NGAL:Cr post-operatively with Hepcidin:Cr ratio at 24 h (AUC 0.84, $p = 0.03$); urinary π-GST post-operatively with urinary Hepcidin:Cr ratio at 24 h (AUC 0.86, $p = 0.01$) and urinary π-GST post-operatively with serum CysC at 24 h (AUC 0.83, $p = 0.03$) (Supplementary Table 3). ROC curves for the three best combinations of biomarkers NGAL:Cr (post-op) — 1.32 × Hepcidin:Cr (24 h); π-GST (post-op) — 0.03 × Hepcidin:Cr (24 h) and π-GST (post-op) + 87.35 × CysC (24 h) are shown (Figure 1).

Biomarker combinations demonstrated increased discrimination of AKI by uncategorized NRI against the prediction provided by the best individual biomarker in the combination (Table 3); for combinations involving 24 h urinary hepcidin, this was predominantly by enhancement of the ability to exclude AKI (significant NRI for non-events only). While proportion of patients with probability of AKI reclassified in the correct direction appears good (NRIs 0.45–1.02 out of a maximum 2.00), the absolute change in probability was low with IDI ranging from 0.06 to 0.16 for biomarker combinations (Table 3). Furthermore, when the clinical utility of the combinations was assessed by examining ability to categorize higher or lower risk of AKI using the optimal cut-off value of the combination compared against the cut-off value of the best individual biomarker, the two-category NRI was not significantly different from zero.

When we assessed the predictive ability of all biomarkers in CART analysis, only NGAL:Cr post-operatively and Hep:Cr at 24 h remained in the final pruned decision tree (Figure 2). Terminal nodes regression tree identified high, medium and low risk of AKI groups in our cohort and in particular enabled classification of a high risk group immediately post-operatively and a very low risk group at 24 h after surgery (Figure 2). However reducing the decision tree to binary classification (high & intermediate vs. low risk) resulted in sensitivity and specificity similar to that at cut-offs of the best linear combinations of biomarkers and non-significant NRI compared to Hepcidin:Cr 24 h alone (Figure 2, Table 3).

Discussion

Summary of findings

Individual biomarkers alone had only fair ability to predict RIFLE-R defined AKI after cardiopulmonary bypass. However, some markers (hepcidin and CysC) performed better at predicting more severe AKI (RIFLE ≥ I). Certain pairs of biomarkers (urinary hepcidin at 24 h or serum CysC at 24 h combined with urinary NGAL at 6 h or urinary π-GST at 6 h) produced significantly improved combined ROC-AUCs and achieved values above 0.8. The best combinations involved one biomarker of tubular injury (NGAL or π-GST) and one biomarker glomerular filtration rate (GFR) (Hepcidin or CysC) and/or one biomarker measured early, and one later in the post-operative course. This suggests combination of markers of different aspects and stages in the pathophysiology of AKI improves prediction. However, combinations did not significantly improve reclassification of risk category. CART analysis identified a higher cut-off for 6 h NGAL:Cr as defining a high risk of AKI while, in those without early elevated NGAL, a higher level of hepcidin at 24 h was effective at excluding AKI, suggesting that CART analysis has the potential for more efficient and clinically applicable diagnostic modeling.

Relationship to previous biomarker research

Moderate diagnostic performance of urinary NGAL and L-FABP in this cohort is in keeping with previous studies in similar groups of adults after CPB.[36,37] Urinary NGAL remained associated with AKI at the 24 h time-point, in keeping with reported performance an older patient population and associated chronic kidney disease.[38] Results regarding hepcidin changes and AKI in this cohort confirmed in two other studies of post-CPB AKI.[39,40]

Finally, CysC has been shown to predict CPB-AKI very early in the post-operative course[41] with similar predictive value to that shown in this study.

Combinations of AKI biomarkers have been described in a several publications.[42–44] Han et al.[43] showed that a combination of proximal tubular injury biomarkers kidney injury molecule 1 (KIM-1) and *N*-acetyl-β-D-glucosaminidase (NAG) had no additive ability to predict AKI in children undergoing CPB. The same authors demonstrated that combination of three biomarkers (KIM-1, NAG and NGAL) was significantly improved prediction or early AKI at 3 h after cardiac surgery in the however, there was no added value for diagnosis of *late* AKI (>24 h).[44] A combination of L-FABP and NAG at 4 h after surgery was associated with an improved ROC-AUC for the prediction of AKI and addition to a clinical risk prediction model produced a further significant increase in AUC.[42] Most recently the ability of 32 potential urinary AKI biomarkers to predict worsening of AKI or death, was studied in 95 patients with mild AKI after cardiac surgery. In this study a combination of KIM-1 (a tubular damage marker) and IL-18 (an inflammatory mediator) was most predictive of death or advanced AKI.[45]

Strengths and limitations

As a small single centre study conclusions regarding clinical risk classification for AKI are limited. However, it is similar in size to other examining multiple combinations of AKI biomarkers and makes important points about the biological nature of complementary biomarkers and the potential disconnect between statistical and clinically significant improvement in risk prediction. We employed a wide panel of candidate biomarkers and measured biomarkers at two post-operative time points. Predictive ability of biomarkers was only fair, but was comparable to many studies in similar patient populations, including many older patients with patients with CKD. This study was restricted to post-CPB AKI and findings may not be generalizable. However, post-CPB AKI is a clinically relevant setting for the investigation of biomarkers of AKI. We only used creatinine-normalized values for urinary biomarkers where recommended by the developers. However, normalization did not significantly improve ROC-AUC of individual biomarkers and the validity of normalization against urinary creatinine has been questioned.[46,47] Our clinical trial patient population was pre-selected for high risk of AKI this provided a high event rate to permit analysis of biomarkers in combination in a small overall sample; however as patients were selected for AKI risk factors it was not designed to permit analysis of biomarkers in combination with clinical risk prediction models.

Statistical analysis of combined diagnostic predictors is complex. Our analysis is strengthened by use of a robust method to combine biomarkers, weighted linear combination. Use of an uncategorized NRI has been criticized as oversensitive and self-fulfilling.[48] However, at least it is likely to be an indicator that certain combinations of biomarkers provide complementary information and thus potential insights into the pathogenesis of AKI. Moreover, this analysis was tempered by examination of the two-category NRI, a better measure of clinical utility. CART models can capture non-linearity much more easily than regression models, are very well suited to predictive modeling and are easy to interpret, however they are prone to over-fitting and results can be variable

depending on the dataset used to develop the tree. To address this concern our model was subject to pruning, a form of internal cross-validation to minimize over-fitting. The model performed similarly to linear combination of predictors when considered as a binary outcome, supporting our primary finding with an alternative methodology. Any model developed from a single sample set does require external validation and the CART model is presented to highlight the potential of this methodology to develop more clinically applicable models for examining multiple biomarkers and/or clinical risk factors in combination and to support our primary analysis.

Finally while certain biomarker combinations resulted in better assignment of risk of AKI practical ability to better categorize groups high and low risk of AKI did not eventuate. However, serum creatinine has significant limitations as a gold standard for diagnosis of renal tubular injury due to alterations in creatinine production, volume of distribution in acute systemic illness and the indirect relationships between tubular injury, GFR changes and alteration in serum creatinine. An imprecise gold standard will intrinsically limit the performance of even the best biomarkers[49] and thus might prevent meaningful improvement of prediction when they are examined in combination. One way to escape this limitation is to examine creatinine independent outcomes such as need for RRT and/or mortality.[50,51] In this study some individual biomarkers (urinary hepcidin at 24 h, urinary NGAL at 24 h and serum CysC at 24 h) did predict need for RRT or death, however as the event rate was small statistical analysis of combinations was not feasible.

Conclusions

AKI biomarkers may aid in the early diagnosis of AKI, however performance in this groups of high-risk adult patients was, at best, fair. Combinations of biomarkers are able to increase diagnostic discrimination, but this may not result in significantly better reclassification of risk of AKI when applied as a simple diagnostic cut-off. Biomarkers that indicate tubular injury and those that may be related to functional changes in GFR may be most informative in combination and should be the target of future research.[52]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Declaration of interest: This study has been supported by Austin Health ICU Research Fund. Dr MW is a shareholder, President and CEO of Intrinsic LifeSciences, developer and distributor of an ELISA assay for hepcidin. Dr VA is a shareholder and officer of Intrinsic LifeSciences. Dr JRP and Professor RB are named in a US patent in conjunction with Dr MW and Prof PD is a co-inventor on NGAL patents for the diagnosis of acute kidney injury. SB, PD, JP and RB have received consulting/speaking fees from Alere Inc. RB has received consulting fees from Abbot Diagnostics Inc. JRP is responsible for statistical design and analysis.

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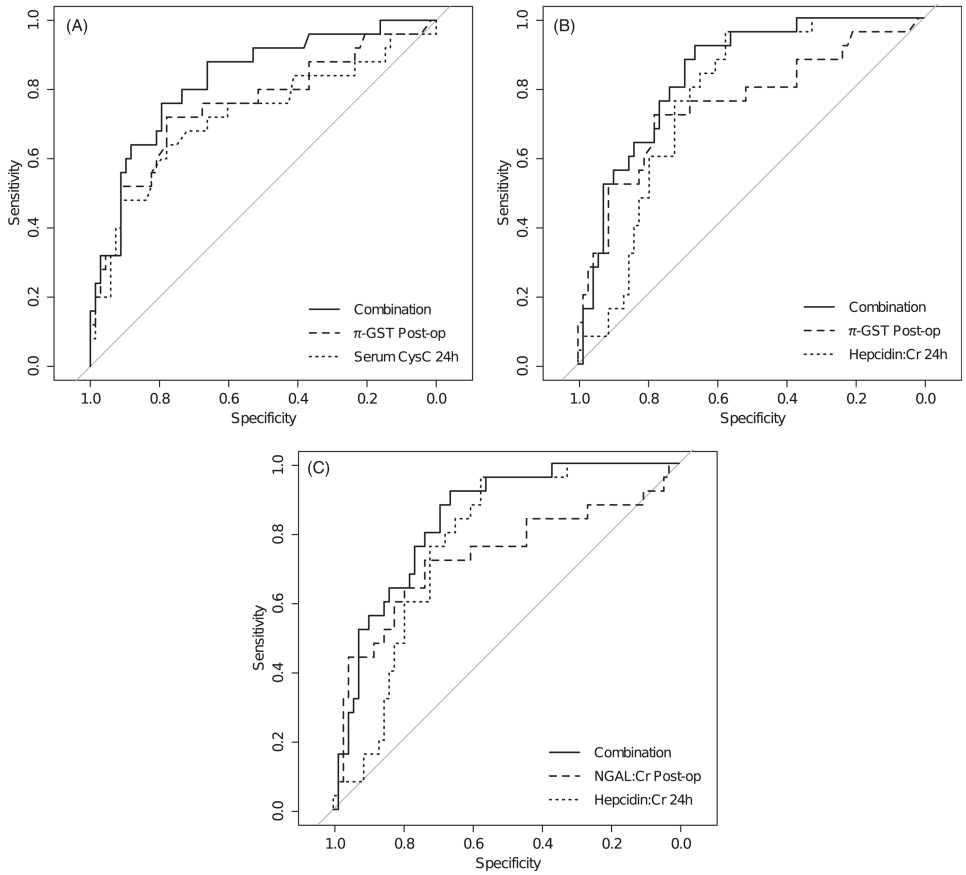

Figure 1.

ROC curves for three AKI biomarker combinations with statistically significant increase in AUC. (Panel A) urine π-GST post-op and serum Cystatin-c at 24 h; (Panel B) urine π-GST post-op and urine Hepcidin:Creatinine at 24 h; (Panel C) urine NGAL:Creatinine post-op and urine Hepcidin:Creatinine at 24 h. We considered linear combinations of biomarkers in the form: $C = B1 + \alpha \times B2$ and determined the value of α (range $-\infty$ to $+\infty$) that provided maximal ROC-AUC for the ability of C to predict AKI. Only combinations of structural biomarkers (NGAL, π-GST) and filtered substances (Hepcidin, CysC) demonstrated statistical significance suggesting markers assessing different aspects of the pathophysiology of AKI may have greater combined diagnostic value.

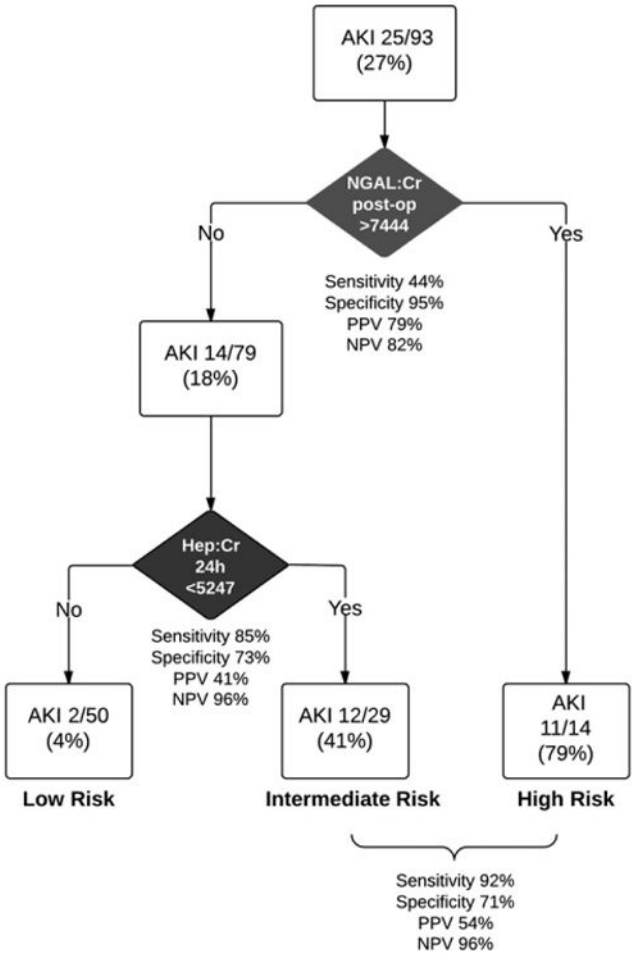

Figure 2.

Classification and Regression Tree (CART) analysis for biomarkers in combination. All biomarkers were included but only NGAL:Cr post-op and Hepcidin:Cr at 24 h remained in the final model. Terminal nodes identified high, medium and low risk groups for AKI. This model allows early identification of a high-risk group immediately post-operatively and then a low risk classification at 24 h.

Table 1

Patient characteristics.

Variable	All	AKI (*n*=25)	No AKI (*n*=68)	*p*
Baseline				
Age (y)	70 (61–76)	72 (67–78)	70 (61–75)	0.14
Female sex	31%	32%	30%	1.0
Pre-operative creatinine	91 (76–113)	86 (72–111)	95 (78–119)	0.29
Chronic kidney disease stage 3	29%	24%	31%	0.61
Chronic kidney disease stage 4	8%	16%	4%	0.08
Insulin-requiring diabetes	7%	8%	7%	1.0
previos cardiac surgery	16%	20%	15%	0.53
L ventricular ejection fraction <35%	6%	16%	3%	0.04
Surgery				
Coronary bypass grafts	59%	76%	53%	0.06
Valve replacement or repair	62%	68%	60%	0.63
Coronary bypass grafts + valve	25%	48%	16%	0.003
Thoracic aortic surgery	11%	20%	7%	0.13
Bypass time (min)	139 (11–202)	210 (146–240)	125 (106–177)	<0.001
Post-operative				
APACHE III score	50 (41–57)	52 (47–69)	47 (40–56)	0.003
% Blood transfusion in theatre or first 24 h	41%	56%	35%	0.096
Any Vasopressor in first 24 h of ICU	48%	58%	44%	0.35
Any Inotrope in first 24 h of ICU	41%	44%	40%	0.81
Fluid balance (first 24 h post-op)	+190 (−886 to +1366)	+43 (−640 to +726)	+310 (−103 to +723)	0.73

Notes: Characteristics of 93 patients undergoing cardiopulmonary bypass by AKI-risk category. Continuous variables are expressed as medians (interquartile range), for categorical variables 95% confidence intervals are shown.

Table 2

ROC AUC analysis for a panel of biomarkers of AKI-risk.

Biomarker	No AKI *n* = 68	AKI *n* = 25	*p* (Univariate)	AUC	AUC 95% CI	Cut-off	Sens (%)	Spec (%)	*p* (AUC)
Pre-op									
NGAL pre-op (ng/mL)	20.7 (5.7–68.3)	17.2 (6.7–62.5)	0.66						
NGAL/Cr pre-op (ng/mg)	31.3 (8.4–100.4)	18.3 (5.3–40.7)	0.20						
L-FAB pre-op (ng/mL)	1.0 (0.53–2.8)	2.1 (0.67–6.3)	0.09						
α GST pre-op (ng/mL)	6.1 (2.4–11.8)	3.8 (1.7–7.5)	0.17						
π GST pre-op (ng/mL)	9.4 (4.3–25.3)	11.4 (3.7–17.1)	0.63						
Hepcidin pre-op (ng/mL)	481 (172–1053)	495 (132–961)	0.73						
Hepcidin/Cr pre-op (ng/mg)	608 (295–1139)	427 (262–1008)	0.31						
Serum CysC pre-op (mg/L)	1.2 (1.0–1.4)	1.2 (1.1–1.4)	0.51						
Post-op									
NGAL post-op (ng/mL)	135 (39–136)	383 (197–1632)	0.002*	0.71	0.59–0.83	>195	76.0	60.3	0.001*
NGAL/Cr post-op (ng/mg)	970 (312–2597)	4709 (1289–9105)	<0.001*	0.73	0.60-0.86	>2346	72.0	73.5	<0.001*
L-FAB post-op (ng/mL)	19 (3–75)	83 (18–143)	0.005	0.69	0.57–0.81	>9.5	88.0	41.2	0.003*
α GST post-op (ng/mL)	6.1 (2.6–11.5)	8.6 (3.6–25.2)	0.14	0.60	0.46–0.74	–	–	–	0.07
π GST post-op (ng/mL)	27.3 (6.6–67.7)	60.6 (148–336)	<0.001*	0.75	0.63-0.88	>71.6	72.0	77.9	<0.001*
Hepcidin post-op (ng/mL)	628 (214–1498)	698 (302–1470)	0.80	0.52	0.38–0.65	–	–	–	0.40
Hepcidin/Cr post-op (ng/mg)	5769 (2883–11,016)	3859 (2398–9971)	0.37	0.52	0.38–0.66	–	–	–	0.37
Serum CysC post-op (mg/L)	1.1 (1.1–1.3)	1.4 (1.2–1.5)	0.005	0.69	0.56-0.82	>1.24	76.0	63.2	0.003*
24 h									
NGAL 24 h (ng/mL)	80 (35–159)	231 (63–399)	0.02	0.65	0.52–0.79	>207	52.0	82.4	0.011
NGAL/Cr 24 h (ng/mg)	79 (33–246)	68 (200–1206)	0.004	0.70	0.57–0.82	>126	64.0	69.1	0.002*
LFAB 24 h (ng/mL)	12.4 (6.8–24.8)	17.5 (6.1–2.2)	0.35	0.56	0.42–0.71	–	–	–	0.17
α GST 24 h (ng/mL)	7.0 (3.2–11.8)	5.2 (2.2–9.6)	0.26	0.57	0.44–0.71	–	–	–	0.13
π GST 24 h (ng/mL)	27.9 (11.7–54.4)	3.4 (15.6–62)	0.17	0.59	0.45–0.74	–	–	–	0.09
Hepcidin 24 h (ng/mL)	8581 (2610–13,492)	2881 (901–4815)	<0.001*	0.73	0.62–0.84	<7855	92.0	52.9	0.0004*
Hepcidn/Cr 24 h (ng/mg)	7935 (4484–11,061)	3846 (2650–5243)	<0.001*	0.77	0.67–0.86	<7313	96.0	57.4	<0.001*
Serum CysC 24 h (mg/L)	1.2 (1.1–1.5)	1.7 (1.3–2.1)	0.001*	0.72	0.59–0.85	>1.57	64.0	77.9	<0.001*

Notes: Prediction of AKI-risk in 93 patients in the first 5 days following CPB. *p* (Univariate): *p* value for univariate comparison AKI versus No AKI (Mann–Whitney *U* test). *p* (AUC): *p* value for ROC-AUC versus AUC = 0.5.

* Remained significant at $p < 0.05$ level after correction for 16 multiple comparisons (Bonferroni).

Table 3

Linear combinations of biomarkers and decision tree analysis.

			Uncategorized NRI				IDI				Best cut-off			2-Category NRI			
Combination	**AUC**	***P***	**Total**	***P***	**Events**	**Non-events**	**Total**	***P***	**Events**	**Non-events**	**Value**	**Sens.**	**Spec.**	**Total**	***P***	**Events**	**Non-events**
π-GST (post-op) – 0.03 × HepcidimCr (24 h)	0.86	0.01	0.57	0.01	0.04	0.53	0.15	0.015	0.11	0.04	>-129	92%	69%	0.08	0.24	-0.04	0.12
NGAL:Cr(post-op) – 1.32 × HepcidimCr (24 h)	0.84	0.03	0.45	0.05	0.04	0.41	0.09	0.06	0.07	0.02	>-5824	92%	66%	0.05	0.45	-0.04	0.09
π-GST (post-op) + 87.35 × CysC (24 hr)	0.83	0.03	1.02	<0.001	0.52	0.50	0.06	<0.001	0.05	0.02	>197	76%	79%	0.05	0.49	0.04	0.015
Classification and Regression Tree analysis: NGAL:Cr(post-op)>7444 OR Hepcidin:Cr (24 h) <5247												92%	71%	0.09	0.12	-0.04	0.13

Note: Biomarkers assessed by ability of ROC best cut-off to classify high or low risk of AKI, reclassification compared to better of the two individual markers.