A preoperative multimarker approach to evaluate acute kidney injury following cardiac surgery

Short title: Acute kidney injury in cardiac surgery

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None.

ABSTRACT

Objective(s): To investigate whether a multimarker strategy combining preoperative biomarkers representing distinct pathophysiological pathways enhances preoperative risk assessment of acute kidney injury following cardiac surgery (CSA-AKI) and elaborates knowledge of underlying pathogenesis.

Design: Prospective, cohort study.

Setting: Single-center tertiary referral hospital.

Participants: 1,015 adults undergoing cardiac surgery with cardiopulmonary bypass.

Interventions: CSA-AKI was defined as ≥ 50% increase in serum creatinine concentration, absolute increase ≥ 26 µmol/l or new requirement for dialysis. Pre- and perioperative information until hospital discharge was registered. Preoperative plasma levels of C-reactive protein, terminal complement complex, neopterin, lactoferrin, N-terminal pro-brain natriuretic peptide (NT-proBNP) and cystatin C were determined using enzyme immunoassays. Biomarkers were selected based on causal hypotheses of underlying mechanisms and were related to inflammatory, hemodynamic or renal signaling pathways.

Measurements and Main Results: 100 patients (9.9%) developed CSA-AKI. Higher baseline plasma concentrations of neopterin and NT-proBNP were independently associated with CSA-AKI ($p=0.04$ and $p<0.001$, respectively). Lower baseline plasma lactoferrin concentrations were observed in patients with CSA-AKI ($p=0.05$). Compared to clinical risk assessment, addition of these biomarkers provided a slight, but significant increment in predictive utility (area under the curve 0.81-0.83, likelihood ratio test $p<0.001$). A net of 12% of patients were correctly reclassified, and improved prediction was especially seen in patients with intermediate risk (56% correct reclassification).

Conclusions: Preoperative hemodynamic, renal and immunologic function play central roles in the pathogenesis of CSA-AKI. Our findings add evidence to the potential of a multimarker approach in order to improve preoperative prediction of CSA-AKI.

Key words: Acute kidney injury, cardiac surgery, preoperative biomarkers, risk prediction

INTRODUCTION

The multifactorial pathogenesis of cardiac-surgery associated-acute kidney injury (CSA-AKI) is related to a complex interaction between baseline predisposition, hemodynamic disturbances, nephrotoxic insults and inflammatory responses. Several prediction models have been developed, combining preoperative clinical characteristics to identify patients at risk of CSA-AKI.[1] In the clinical setting, preoperative prediction of CSA-AKI may facilitate patient consulting, clinical decision-making and commencement of primary preventive strategies, before significant damage has taken place (Figure 1). Furthermore, it may facilitate a fair allocation of hospital resources. For research purposes, there has been a call for novel interventions in the early prevention of AKI in high-risk patients, and preoperative risk modeling may both contribute to enhanced identification of at-risk patients eligible for trial-specific intervention as well as recognition of central pathways and thus potential treatment targets.

However, it has been shown that existing models are of insufficient precision with a tendency of underestimating the AKI risk.[2, 3] Furthermore, most investigators have used dialysis-dependent AKI or severe AKI as their endpoint, whereas even smaller changes in renal function are associated with cardiovascular adverse events and reduced survival.[4-6]

The limited ability to identify patients who develop CSA-AKI may indicate that some patients have a subclinical vulnerability to AKI that is not predictable through clinical risk factors alone. We hypothesized that there exist preoperative biomarkers more closely related to the baseline risk that may improve our ability to predict CSA-AKI. Some preoperative biomarkers (natriuretic peptides, cystatin C) have already been suggested to provide additional or superior information about the risk for AKI following adult cardiac surgery.[7, 8] However, a single biomarker may not provide sufficient precision because of the multifactorial pathogenesis and heterogeneous patient populations. Therefore, we hypothesized that a multimarker approach reflecting different potentially pathogenic mechanisms, including inflammatory, hemodynamic or renal signaling pathways, could provide complementary information, improve risk stratification and increase our understanding of the complex pathology behind CSA-AKI. Thus, the aim of this prospective study was to investigate the associations of preoperative plasma C-reactive protein (CRP), terminal complement complex (TCC), neopterin, lactoferrin, N-terminal pro-brain natriuretic peptide (NT-proBNP) and cystatin C with the risk of AKI following cardiac surgery.

METHODS

The study was part of the Cardiac Surgery Outcome Study (CaSOS). Approval was given by The Norwegian Data Inspectorate and The Regional Research Ethics Committee in Medicine (Project number 4.2007.1528), Trondheim, Norway on 27.06.2007. Written informed consent was obtained from all patients.

Consecutive adult patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) at St. Olavs University Hospital in Trondheim, Norway, from 04.01.2008 through 04.19.2010 were enrolled. Patients on preoperative dialysis or with missing preoperative serum creatinine data were excluded. All exclusions are outlined in Figure S1, Supplementary Methods 1 (SM1), leaving 1,015 patients for analysis. Information on patient and procedural characteristics, laboratory tests, and postoperative factors until hospital discharge was prospectively registered as part of the department's quality assurance work. Plasma from preoperative blood samples was stored at -80˚C for later analysis. The same study cohort was included in three previous investigations from CaSOS.[9-11]

The end-point was postoperative AKI defined as ≥ 50% increase after surgery from the baseline serum creatinine concentration, an absolute increase ≥ 26 µmol/l or a new requirement for dialysis, including all stages of AKI as defined by the *Kidney Disease: Improving Global Outcomes* (KDIGO)-guidelines.[12] A slight modification from the original definition was used, as data on urine output was unavailable and we allowed a longer time span than 48 hours for an absolute increase or 7 days postoperatively for a doubling in serum creatinine concentration to occur. Changes in serum creatinine from baseline were based on the maximum postoperative concentration, as previously employed in CaSOS.[13]

Plasma biomarkers

Biomarker selection was based on causal hypotheses. We hypothesized that inflammatory, hemodynamic and renal signaling pathways are central mechanisms in the preoperative increased risk for CSA-AKI, and biomarkers for these pathways were chosen (Table 1).

Preoperative plasma concentrations of CRP, neopterin, TCC, lactoferrin, cystatin C and NT-proBNP were analysed using enzyme immunoassays. High-sensitivity CRP, TCC, neopterin and lactoferrin were estimated as earlier described.[11] NT-proBNP and Cystatin C were measured using commercial kits (Human NT-proBNP ELISA Kit, MyBioSource, Inc., San Diego, CA, USA; RayBio Human Cystatin C ELISA, RayBiotech Inc., Norcross, GA, USA; and BioVendor Human Cystatin C ELISA, Biovendor Research and Diagnostic Products, Brno, Czech Republic).

Statistical analysis

Data are described using median with 95% confidence intervals (CI) or frequencies with percentages, as appropriate. Outcome comparisons were performed with the Mann-Whitney U-test or Chi-square test. Pearson's correlation coefficients were used to test linear correlation between biomarkers, where coefficients between 0.1-0.3, 0.3-0.6 and >0.6 were considered to indicate weak, moderate and strong correlation, respectively. Statistical analyses were performed using Stata (version 13.1, StataCorp LP, Lakeway Drive, USA), R (version 3.2.2, Foundation for Statistical Computing, Vienna, Austria) and Minitab 17 (Minitab Ltd, Coventry, UK).

Associations between biomarkers and the incidence of CSA-AKI were investigated using an explanatory approach,[21] following a 3-step procedure applying logistic regression: (1) Unadjusted analyses of each biomarker separately; (2) Simultaneous testing of all biomarkers; (3) Multivariate analyses adjusting for relevant preoperative clinical predictors.

Biomarkers

The linearity assumption was tested by plots and splines. Biomarkers were natural log-transformed when appropriate. NT-proBNP was dichotomized with a cut-off value >125 pg/ml as recommended for preoperative screening in moderate-to-high risk patients.[22, 23] Biomarkers with p-value <0.10 were included into the multivariate analyses.

Clinical variables

The purpose of the clinical model was to represent clinical risk factors appropriately and adjust for them in order to explore underlying independent associations of biomarkers predicting CSA-AKI. To avoid spurious results we compared four different approaches applying (1) the clinical predictors derived previously in our centre (CaSOS' CSA-AKI model);[13] (2) the additive Cleveland clinical risk score;[24] (3) the UK any-stage AKI risk calculator;[25] and (4) by constructing a parsimonious model based on novel hypotheses of mechanisms. The Cleveland clinical risk score was originally developed to predict dialysis-dependent AKI, but has proven applicable at different severity levels of CSA-AKI.[26, 27] The different models are outlined in Table S1 in Supplementary Methods (SM1).

For novel model development, the selection of potential clinical predictor variables was guided by an extensive literature review and clinical knowledge, as recommended to avoid overfitting and confounding with selection based on univariate analyses.[28, 29] Backward limited stepdown was performed and the final predictors were internally validated by bootstrap resampling (200 runs). As a sensitivity analysis, alternative models with different indicators of pre-existing renal dysfunction (serum creatinine, creatinine clearance, or estimated GFR based on creatinine or creatinine and cystatin C combined[20]) and heart function (ejection fraction, diagnosis of chronic heart failure or New York Heart Association (NYHA) Functional Classification) were tested. Models were checked for linearity, predefined interactions and overly-influential observations. Model discrimination was evaluated using the area under the receiver-operating characteristic curve (AUC). Goodness-of-fit was assessed with a plot of observed vs. predicted outcomes and Hosmer-Lemeshow (HL) test. Predicted performance of the final model in future data sets was evaluated using the estimated shrinkage factor and a calibration plot.

Final model selection was based on comparison of the AUC, HL test, log likelihood, Akaike and Bayesian information criteria (AIC and BIC) (section 1.3 in SM1).

Multivariate analysis

Finally, we analyzed whether the biomarkers remained significant predictors of CSA-AKI when adjusting for clinical variables. Predefined interactions between covariates were tested. As a sensitivity analysis, biomarkers were adjusted with each of the clinical models in order to assess the robustness of the associations and reduce the risk of overfitting. The model including novel biomarkers was compared to the clinical model without biomarkers by a likelihood ratio test. Final estimation was performed on 1,005 patients after excluding eight patients (0.8%) with incomplete data for all biomarkers and two overly-influential cases. Model estimates are presented with bootstrapped confidence intervals (200 runs). Estimated risk levels were plotted as predicted probabilities across decile groups of predicted risk with the observed proportions of AKI cases superimposed. Model discrimination and goodness-of-fit was evaluated as described above.

The incremental contribution of the biomarkers to the final clinical model was assessed by improvements in AUC, the integrated discrimination improvement (IDI) and the continuous and categorical net reclassification improvement (NRI) (for a more detailed explanation, see section 1.4 in SM1). In order to evaluate the effect of the biomarkers on AKI risk classification, patients were grouped into low (< 10%), intermediate (10-20%) and high (>20%) risk groups according to their calculated AKI risk by the clinical model before addition of the biomarkers. Pencina and colleagues have proposed that in situations with no established cut-offs, using the event rate as the default risk threshold may not be unreasonable,[30] which was therefore done in the present study. The NRI was calculated as the sum of improvement for cases and controls. Due to lack of clear risk thresholds for CSA-AKI, we also calculated the “continuous NRI” or NRI(>0). The continuous NRI has been recommended in situations where the primary focus is on the strength of the marker rather than model performance.[31] A continuous NRI >0.6, around 0.4 and <0.2 are considered strong, intermediate and weak, respectively.[32]

RESULTS

100 patients (9.9%) developed postoperative AKI following cardiac surgery. Patients with CSA-AKI were older and more frequently presented with diabetes, hypertension, chronic pulmonary disease and peripheral vascular disease (Table 2). AKI cases more often had pre-existing left ventricular hypertrophy or chronic cardiac failure; however, they did not present with lower ventricular ejection fractions. They had significantly higher baseline serum creatinine concentrations. Patients suffering CSA-AKI more often had a history of previous cardiac surgery. They generally underwent more complex surgical procedures, spent longer time on CPB and more frequently received intraoperative inotropic support. Detailed comparisons of AKI cases and controls are provided in Table 2.

Biomarkers

Baseline concentrations of fluid-phase markers are given in Table 3. Neopterin, CRP, cystatin C and NT-proBNP concentrations were higher ($p<0.001$ for all) in patients developing CSA-AKI. Lactoferrin concentrations were lower in AKI cases, but this difference did not reach statistical significance ($p=0.05$). Neopterin, lactoferrin, NT-proBNP and cystatin C remained significant in a simultaneous test of all biomarkers. Correlations amongst the different markers were weak (Table S2a in Supplementary Material 2 (SM2)).

Clinical variables

The final parsimonious clinical model used to adjust for biomarkers in the multivariate analysis comprised age, body mass index above 30 kg/m^2, female gender, multiple surgical procedures and preoperative serum creatinine level. By overall judgement, this model provided better fit than any of the previously published prediction models for CSA-AKI (Table S3a in SM2): Besides being simpler, it showed lower AIC, intermediate BIC, high discrimination (AUC 0.800 (95% CI 0.758-0.842)) and excellent goodness-of-fit (HL test, $p=0.47$). Alternative models from the sensitivity testing including data on chronic heart failure, NYHA class, diabetes, pulmonary disease or urgency level of operation did not improve model performance (data not shown).

Multivariate analysis

Neopterin, NT-proBNP and lactoferrin emerged as independent predictors of CSA-AKI (Table 3). Cystatin C was moderately correlated with serum creatinine levels (R=0.48), but did not provide significant information above that of preoperative creatinine concentrations (p=0.15). Substitution of creatinine with cystatin C did not improve model performance. However, when estimating GFR using the combined creatinine-cystatin C equation, the model showed somewhat better fit (Table S4 in SM2). Thus, in the final model, estimated GFR based on serum cystatin C and creatinine concentrations substituted serum creatinine concentration alone (Table 4).

Neopterin was correlated with serum creatinine (R=0.56), however neopterin remained significant also after adjusting for kidney function (neopterin/creatinine ratio). This adjustment did not alter any results, thus the parsimonious model without adjustment was kept. In well-calibrated clinical models, NT-proBNP and neopterin consistently emerged as independent predictors of CSA-AKI (Table S3b in SM2). Initially, increased serum lactoferrin concentrations were associated with a protective effect on CSA-AKI (Table 3); however, this association was no longer significant after bootstrapping the estimates in the final model with adjustment for clinical variables (Table 4, p=0.08).

Addition of baseline NT-proBNP, lactoferrin and neopterin levels to the clinical variables improved model fit (Table 4, LR test p<0.001). The final model showed a median predicted CSA-AKI risk of 6.3% (95% CI 5.8-7.0%, range 0.3%-81.3%) (Figure 2). AKI-cases showed a median predicted risk of 21.5% (95% CI 17.1-26.1) in the baseline model, and 27.2% (95% CI 22.0-32.3) in the combined model. In comparison, patients without CSA-AKI had median predicted risks of 6.1% (95% CI 5.7-6.6) and 5.3% (95% CI 4.8-5.8) based on the baseline and combined models, respectively.

The continuous NRI was 0.55 (0.34-0.75). When comparing the effect of including biomarkers on AKI risk categories, a net 12% of all patients were reclassified correctly when combining

biomarkers and clinical variables (categorical NRI 0.12, p=0.05, section 2.4 in SM2). 10% were due to AKI-patients being correctly reclassified to higher risk groups (Table S5 in SM2). A subgroup analysis was performed in the intermediate risk group (predicted risk 10-20%, n=175 (17%)). Among AKI cases (n=22), 11 patients were correctly upgraded in risk category, whereas 5 incorrectly downgraded, yielding a net correct reclassification in 6 out of 22 AKI-patients (27%). Correspondingly, among non-AKI cases (n=153), 71 patients were correctly downgraded, whereas 26 incorrectly put into a higher risk category, yielding a correct net reclassification of 45 (29%) non-AKI cases. The overall NRI in the intermediate group was therefore 56%.

The combined risk model demonstrated excellent agreement between predicted and observed risks (Figure 2, HL test p=0.87). There was an incremental increase in the AUC from 0.806 (95% CI 0.764-0.847) with clinical variables only, to 0.832 (95% CI 0.791-0.873, p=0.05) when including biomarkers. The improvement in prediction seen in the intermediate risk group, as demonstrated with reclassification, was also confirmed by the AUC graph (Figure 3).

DISCUSSION

In the present study, neopterin and NT-proBNP emerged as independent predictors of CSA-AKI. The statistical significance of the protective effect of higher baseline lactoferrin concentrations was inconsistent, which may be due to the sample size. Cystatin C was not superior to serum creatinine as an indicator of preoperative renal function. However, estimated GFR combining both creatinine and cystatin C provided subtle improvement in model performance. Inclusion of biomarkers provided significant incremental information regarding the risk of CSA-AKI beyond traditional, clinical risk factors. Improved prediction was especially seen in patients with intermediate AKI risk, where a net of 27% of AKI cases and 29% of control patients were correctly reclassified (overall NRI 56%).

We explored biomarkers that may help understand and predict CSA-AKI preoperatively. Whereas later prediction including perioperative variables and biomarkers may enhance risk stratification and identification of injury and impaired renal function,[13, 35] we find that preoperative prediction is of greater clinical utility.

Clinical utility

A comprehensive analysis of the added incremental value of novel predictors confirmed that preoperative information about NT-proBNP, neopterin and lactoferrin significantly enhanced the predictive ability for CSA-AKI. Findings remained robust independent of the clinical risk model used for adjustment, underscoring the independency of the biomarkers from clinical risk factors. The additive role of each biomarker confirmed our hypothesis regarding the importance of including biomarkers from different pathophysiological pathways.

Previous studies on preoperative biomarkers of AKI have only considered high-risk patients.[7, 8] Shlipak and colleagues investigated the impact of preoperative cystatin C on AKI risk prediction, where addition of cystatin C to a baseline clinical model increased AUC from 0.70 to 0.72 ($p<0.01$) and led to a NRI of 21%.[8] Similarly, Patel and coworkers assessed preoperative BNP in prediction of CSA-AKI, where addition of BNP increased AUC from 0.67 to 0.68 (LR test

p=0.01) compared to the baseline, clinical model.[7] The continuous NRI in all patients with AKI was calculated to 0.18. Of note, improved risk prediction was most prominent in controls. The modest effects seen in these single biomarker analyses may indicate that a single biomarker may not provide sufficient precision. A combination of preoperative variables and biomarkers was identified in the present study, which provided an overall better fit compared to previous studies.

Addition of multiple biomarkers to the clinical model increased the AUC from 0.81 to 0.83. It has been shown that increases in AUC are smaller if the baseline model has a high AUC.[30] The likelihood ratio test was significant, which is a better criterion when evaluating the incremental contribution of a new marker.[36, 37] Furthermore, the present data demonstrate a significant impact on reclassification of AKI patients, and a continuous NRI of 0.55 indicating a moderate-to-strong effect size. Assessment of the AUC and NRI both indicated that prediction was particularly improved in patients with intermediate AKI risk, where a purely clinical judgement may be more difficult.

Nevertheless, the focus of this study was not to design a definite prediction model for clinical use, but to assess the importance of a multimarker strategy and the combined strength of the added biomarkers. It has been recommended that studies of an explanatory character report both the explanatory and predictive qualities of the final model, so that it can be fairly evaluated in terms of its capabilities and compared to other models.[21] With the modest increase in AUC and uncertain clinical implications gained by correct reclassification, the cost-effectiveness remains to be demonstrated. The previous failure to identify effective intervention for AKI has placed continued emphasis on optimizing supportive treatment and avoiding secondary renal injury, such as watchful hemodynamic monitoring and restraining from nephrotoxins (Figure 1). However, improved understanding of underlying biology may also facilitate identification and development of novel treatment strategies.

Potential mechanisms

Preoperative cardiac and renal function are well-known predictors of CSA-AKI, where altered function may disturb their intricate regulation of cardiac output, volume status and vascular tone, affecting hemodynamic stability and end-organ perfusion.[38] Furthermore, preoperative prediction becomes complicated by individual variations in the inflammatory response.

Renal function

Preoperative renal function has been pointed out as the most important determinant of CSA-AKI.[39] Nevertheless, estimation methods for renal function are debated. In the present study, plasma cystatin C showed moderate correlation with serum creatinine. However, as opposed to earlier findings,[8] preoperative cystatin C did not enhance prediction of CSA-AKI. Reasons for these conflicting results could be that the previous study was restricted to high-risk patients, and consequently, the incidence of AKI cases was considerably larger (36%).[8] The present inclusion of consecutive cardiac surgery patients and consequent smaller frequency of AKI cases may render smaller differences in alternative measures of renal function more difficult to detect. However, estimating GFR using combined data on creatinine and cystatin C concentrations improved model fit slightly compared to creatinine-based estimates alone, indicating that these markers are indeed complementary. This complies with previous findings from Inker showing that the combined creatinine–cystatin C equation performed better than equations based on either markers alone for detection of chronic kidney disease.[20]

Cardiac function

Patients with a baseline NT-proBNP concentration >125 pg/ml showed a higher risk of CSA-AKI, despite adjusting for relevant clinical disorders. This is in accordance with previous findings,[7, 40, 41] underlining the importance of hemodynamic stress in the pathogenesis of CSA-AKI. NT-proBNP is released into the circulation in situations with volume expansion or pressure overload. Inadequate renal perfusion has traditionally been considered the most important event in the course of CSA-AKI.[39] However, there has been an increasing focus towards the importance of increased venous pressure in causing reduced renal function and a higher risk of

AKI in patients with acute or chronic heart failure.[7] The lack of association with ejection fraction in our study strengthens this view. Furthermore, the lacking association between diagnoses of chronic heart failure or NYHA class with the risk of CSA-AKI underscores the importance of a more objective marker to identify patients at risk.

Inflammation

CPB activates a systemic inflammatory response which is modulated by many factors and processes including tissue injury, blood exposure to the artificial surface of the CPB, ischemia/reperfusion injury, perioperatively administered drugs and hemodilution, as well as individual variations. In the present study, we sought to investigate the role of baseline inflammation by including key mediators of different inflammatory pathways: CRP, TCC, lactoferrin and neopterin represent activation of general inflammation, the complement system, neutrophils and macrophages, respectively. Their different roles were supported by the low correlations among them.

Neopterin emerged as an independent predictor of CSA-AKI. Neopterin has previously been associated with the risk for cardiovascular events,[42] including cardiac dysfunction following cardiac surgery.[11] The underlying mechanisms, however, are not clear. Neopterin is released from activated macrophages and monocytes. Its only weak-to-moderate correlation with CRP may suggest that neopterin more specifically acts as a marker of active atherosclerosis and aggressive cardiovascular disease in cardiac surgery patients.[42] However, neopterin may also be directly related to CSA-AKI. Neopterin increases the generation of radical oxygen species and enhances oxidative stress,[42] and may thus exacerbate the renal insult following cardiac surgery due to ischemia/reperfusion and inflammation caused by intraoperative aortic cross-clamping and CPB.

In the univariate analysis, AKI cases showed lower baseline lactoferrin concentrations. Being released from secondary granules in neutrophils, lactoferrin correlates with the amount and

activity of neutrophils. Lactoferrin possesses a range of functions, including immunomodulation and iron-binding.[43] After adjustment for clinical variables, lactoferrin emerged as a significant AKI predictor with a p-value of 0.04. However, application of bootstrapping techniques to assess the uncertainty of the sample estimates indicated that the influence of lactoferrin must be evaluated in larger datasets.

There may exist other biomarkers that were not investigated in this study. Although the design of the study does not allow us to draw conclusions about underlying mechanisms, the combination of biomarkers underscore preoperative cardio-renal function and inflammatory properties as key pathways for CSA-AKI. The pathogenic pathways may be differently weighted in each individual, highlighting the importance of evaluating multiple biomarkers with distinct pathophysiological backgrounds simultaneously, in order to improve the understanding and prediction of CSA-AKI.

In conclusion, neopterin and NT-proBNP emerged as independent preoperative predictors of CSA-AKI. Higher baseline lactoferrin concentrations may exert a protective effect on CSA-AKI, but further investigation is warranted. Inclusion of the biomarkers into a parsimonious clinical prediction model with age, gender, obesity, surgical category and preoperative renal function provided a significant increment in predictive utility for CSA-AKI. Improved prediction was especially seen in patients with intermediate AKI risk. Further studies are needed to explore whether there exist other useful biomarkers. Findings from the present study underline the importance of a multimarker approach in order to improve preoperative prediction of CSA-AKI.

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FIGURE LEGENDS

Figure 1: Clinical course of acute kidney injury (AKI). Initiation of AKI can involve hemodynamic changes in GFR, sub-clinical tubular injury, or both processes occurring simultaneously. A short time window may exist where specific therapy might reverse AKI; however, this treatment may need to be tailored to the nature of the injury and risk profile of the patient. Established AKI requires days to weeks for recovery, and the emphasis during this period should be on supportive therapy and the avoidance of secondary renal injury that may result in non-recovery of renal function or chronic kidney disease (CKD). These remain the main strategies in this patient group to date. (Figure and legend reproduced with modifications from Prowle JR. Acute kidney injury: an intensivist's perspective. Pediatr Nephrol (2014; 29:13-21.). With permission from Springer.)

Figure 2: Predictiveness curve of the combined model with clinical variables and biomarkers. The excellent goodness-of-fit is demonstrated by the agreement between the black line, showing mean predicted risk of acute kidney injury (left y-axis) along decile groups of predicted risk, and the black dots, indicating the observed risk in the respective groups. The horizontal line shows the overall incidence of acute kidney injury (9.9%). The distribution of predicted risk in the study population (right y-axis) is sketched with a grey, dashed line.

Figure 3: Comparison of the area under the receiver-operating characteristic curve (AUC) of the clinical model without biomarkers, with the model including N-terminal pro-brain natriuretic peptide (NT-proBNP), lactoferrin and neopterin.

Table 1: Overview over the origin, function and application of analyzed biomarkers

Biomarker	Origin	Indicator of	Current clinical application
C-reactive protein	Produced by hepatocytes in response to circulating interleukin-6.	Inflammation; acute-phase-reactant.	Diagnosis and monitoring of inflammatory and infectious diseases, prediction of cardiovascular disease.[14]
Neopterin	Produced by activated macrophages and monocytes.	Inflammation; cellular immune response.	Novel biomarker; suggested in monitoring of inflammatory and infectious diseases.[15]
Terminal complement complex	End product of the complement cascade; assembled by its five late components; C5b-9.	Inflammation; complement activation.	Novel biomarker; suggested in evaluation of biocompatibility of treatments such as CPB.[16]
Lactoferrin	Produced and released by neutrophils.	Inflammation; neutrophil activation.	Novel biomarker; suggested in monitoring of inflammatory and infectious diseases.[17]
NT-proBNP	Released from ventricular myocytes in response to myocardial stretch.	Hemodynamic function; diastolic heart dysfunction and volume overload.	Diagnostics of acute dyspnea[18] and monitoring of heart failure.[19]
Cystatin C	Produced by all nucleated cells at a constant rate.	Renal function; estimated glomerular filtration rate.	Estimation of kidney function and diagnosis of chronic kidney disease.[8, 20]

NT-proBNP; N-terminal pro-brain natriuretic peptide.

Table 2: Patient and perioperative characteristics

	AKI-cases (n=100)	Non-AKI cases (n=915)	*P*-value
Age (years)	74 (71-76)	67 (66-68)	<0.001
Female gender	28 (28.0%)	254 (27.8%)	0.96
Body mass index (kg/m^2)	28.3 (27.3-29.4)	26.9 (26.6-27.2)	0.004
Smoker	45 (45.0%)	513 (56.1%)	0.04
Diabetes mellitus	21 (21.0%)	121 (13.2%)	0.03
Lipid-lowering treatment	69 (69.0%)	763 (83.4%)	<0.001
Hypertension	74 (74.0%)	548 (59.9%)	0.006
ACE inhibitor use	49 (49.0%)	347 (37.9%)	0.03
Left ventricular hypertrophy	38 (38.0%)	203 (22.2%)	<0.001
Chronic cardiac failure[a]	20 (20.0%)	101 (11.0%)	0.009
Ventricular ejection fraction[b]			0.13
1) > 50%	51 (51.0%)	563 (61.5%)	
2) 31-50%	41 (41.0%)	312 (34.1%)	
3) 21-30%	7 (7.0%)	37 (4.0%)	
4) ≤ 20%	1 (1.0%)	3 (0.3%)	
NYHA class III or IV	70 (70.0%)	571 (62.4%)	0.14
Peripheral vascular disease	22 (22.0%)	131 (14.3%)	0.04
Chronic pulmonary disease	27 (27.0%)	115 (12.6%)	<0.001
Endocarditis	1 (1.0%)	2 (0.2%)	0.17
Urgent operation (within 2 weeks)	51 (51.0%)	400 (43.7%)	0.16
Previous cardiac surgery	11 (11.0%)	34 (3.7%)	0.001
Surgical risk groups			<0.001
1) Isolated CABG	35 (35.0%)	609 (66.6%)	
2) 1 procedure non-CABG	13 (13.0%)	114 (12.5%)	
3) 2 surgical procedures	44 (44.0%)	168 (18.4%)	
4) ≥ 3 surgical procedures	8 (8.0%)	24 (2.6%)	
Haemoglobin (g/dl)	13.4 (13.0-13.8)	14.1 (14.0-14.2)	<0.001
Serum creatinine (µmol/l)	98 (92-105)	80 (79-81)	<0.001
Creatinine clearance[c] (ml/min)	71 (65-77)	91 (89-93)	<0.001
Estimated glomerular filtration rate[d] (ml/min/1.73 m^2)	68 (63-73)	88 (86-89)	<0.001

CPB time (min)	117 (106-129)	76 (74-79)	<0.001
Intraoperative inotropic support	42 (42.0%)	203 (22.2%)	<0.001
Intraoperative vasoconstrictor use	97 (97.0%)	876 (96.0%)	0.61
Fluid balance intraoperatively	3048 (2825-3275)	2740 (2695-2783)	0.002
Intraoperative red blood cell transfusion	42 (42.0%)	155 (16.9%)	<0.001
Intraoperative plasma transfusion	39 (39.0%)	110 (12.0%)	<0.001
EuroSCORE II	5.5 (4.7-6.4%)	2.2 (2.1-2.4%)	<0.001
30-day mortality	5 (5.0%)	6 (0.7%)	<0.001

Continuous variables are presented as median (95% CI), categorical variables as n (%). ACE, angiotensin-converting enzyme; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; EuroSCORE, European Risk Stratification Score System; NYHA class, The New York Heart Association Functional Classification (I-IV). Conversion factor for serum creatinine in mg/dl to µmol/l: x 88.4.

[a]The diagnosis of chronic heart failure was based on history and clinical evaluation by an attending cardiologist.

[b]Estimated (see supplementary methods, Supplementary Material 1)

[c]Creatinine clearance calculations based on formula from Cockcroft and Gault.[33]

[d]Estimated glomerular filtration rates calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Creatinine Equation (2009).[34]

Table 3: Preoperative plasma concentrations of biomarkers and logistic regression results[a]

Biomarker	Missing (n)	Baseline serum concentrations		Step 1: Univariate biomarker analysis	Step 2: Multimarker analysis	Step 3: Adjusted multimarker analysis[d]
		AKI-cases (n=100)	Non-AKI cases (n=905)			
Lactoferrin (µg/l)[b]	2	125 (111-149)	149 (142-158)	0.05	0.04	0.05
Neopterin (nmol/l)[b]	6	10.5 (9.4-11.9)	7.4 (7.2-7.6)	<0.001	0.001	0.03
C-reactive protein (mg/l)[b]	2	5.8 (4.4-7.7)	2.5 (2.3-3.0)	<0.001	0.70	0.40
Terminal complement complex (ng/l)	3	188 (168-208)	174 (167-181)	0.47	---	---
Cystatin C (mg/l)[b]	4	1.07 (0.98-1.18)	0.85 (0.83-0.87)	<0.001	0.001	0.15
NT-proBNP (pg/ml)[c]	3	313 (230-488)	108 (99-119)	<0.001	<0.001	0.001

Two overly-influential cases were excluded. Multivariate analyses were performed on cases with complete data on all biomarkers (n=1,005).

[a]P-values at each of the three steps of the statistical analysis (see methods for further details).

[b]Natural log-transformed.

[c]Dichotomized at a cut-off value of >125 pg/ml.

[d]With adjustment for gender, age, body mass index ≥ 30 kg/m^2, surgical category and preoperative serum creatinine.

Table 4: Nested logistic regression analysis comparing the clinical model without and with biomarkers

	Clinical model			Clinical model with biomarkers		
Predictor parameters	OR	95% CI	P-value	OR	95% CI	P-value
Age (per 5 years)	1.19	1.02-1.39	0.03	1.09	0.93-1.27	0.30
Female gender	0.56	0.33-0.96	0.04	0.54	0.31-0.96	0.03
Body mass index >30 kg/m^2	2.12	1.25-3.61	0.006	2.51	1.47-4.28	0.001
Multiple surgical procedures	3.63	2.30-5.73	<0.001	3.22	1.87-5.54	<0.001
Estimated GFR[a] (per 5 ml/min per 1.73m^2)	0.84	0.79-0.89	<0.001	0.91	0.85-0.97	0.006
NT-proBNP >125 pg/ml	---	---	---	2.86	1.63-5.01	<0.001
Neopterin[b]	---	---	---	2.70	1.45-5.02	0.002
Lactoferrin[b]	---	---	---	0.70	0.47-1.04	0.08
Model evaluation parameters						
AUC (95% CI)	0.806 (0.764-0.847)			0.832 (0.791-0.873)		
Akaike information criterion	538.7			510.4		
Bayesian information criterion	568.1			554.6		

Final model parameters with bootstrapped confidence intervals (200 runs). The nested analysis was performed on cases with complete data for all biomarkers (n=1,005). AUC, area under the receiver-operating characteristic curve; CI, confidence interval; GFR, glomerular filtration rate; NT-proBNP, N-terminal pro-brain natriuretic peptide; OR, odds ratio.

[a]Calculations were based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine-cystatin C single equation (2012): $135\times\min(Scr/\kappa, 1)^{\alpha}\times\max(Scr/\kappa, 1)^{-0.601}\times\min(Scys/0.8, 1)^{-0.375}\times\max(Scys/0.8, 1)^{-0.711}\times0.995^{Age}$ [×0.969 if female][×1.08 if black], where Scr is serum creatinine, Scys is serum cystatin C, κ is 0.7 for females and 0.9 for males, α is −0.248 for females and −0.207 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.[20]

[b]Natural log-transformed.

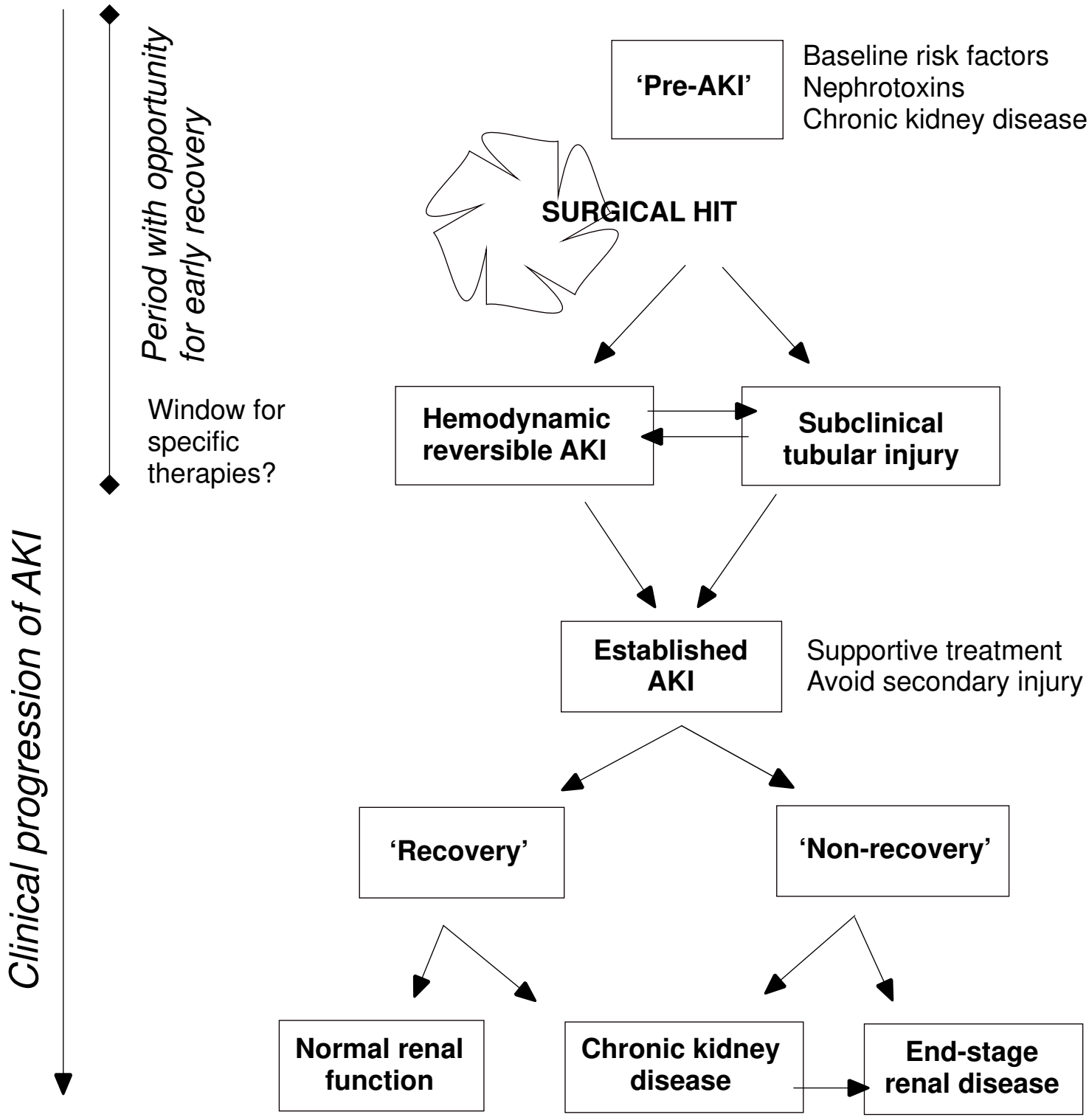
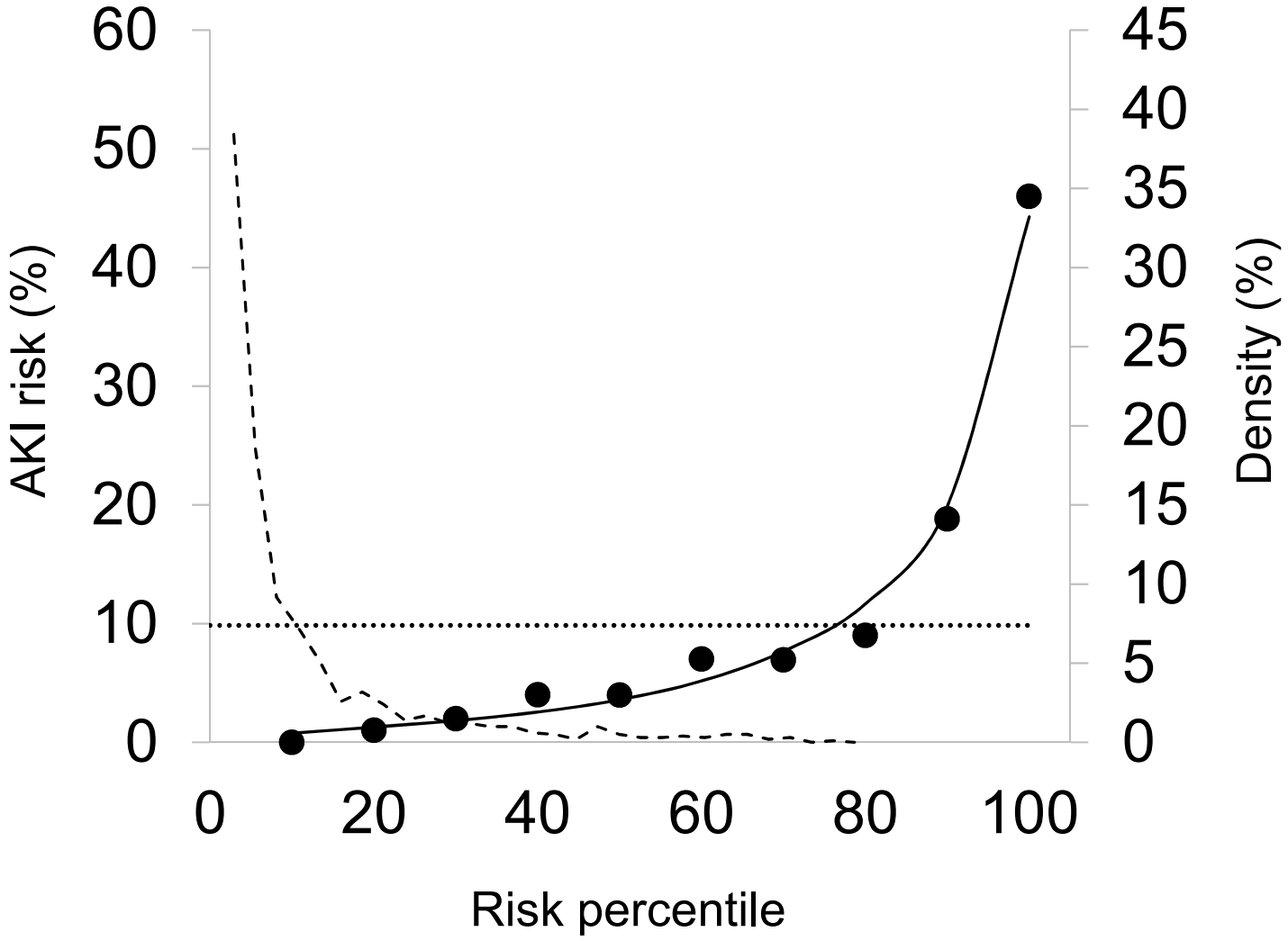

Clinical progression of AKI
Period with opportunity for early recovery
Window for specific therapies?
'Pre-AKI'
Baseline risk factors
Nephrotoxins
Chronic kidney disease
SURGICAL HIT
Hemodynamic reversible AKI
Subclinical tubular injury
Established AKI
Supportive treatment
Avoid secondary injury
'Recovery'
'Non-recovery'
Normal renal function
Chronic kidney disease
End-stage renal disease

Figure 1


AKI risk (%)
60
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Density (%)
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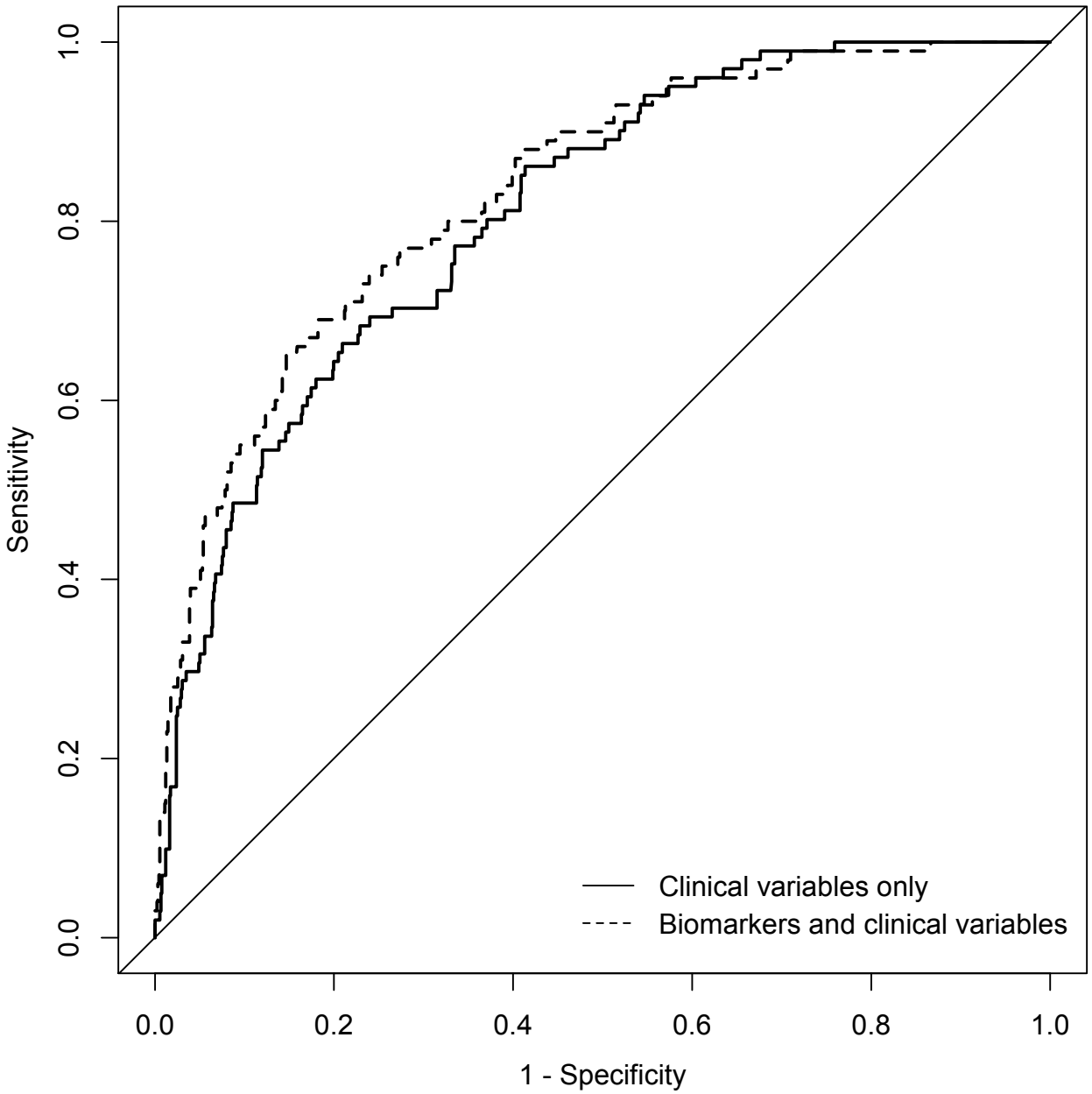
Risk percentile

Figure 2


Sensitivity
1 - Specificity
0.0
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Clinical variables only
Biomarkers and clinical variables

Figure 3

Supplementary Material 1: Supplementary methods

1.1 Patient selection

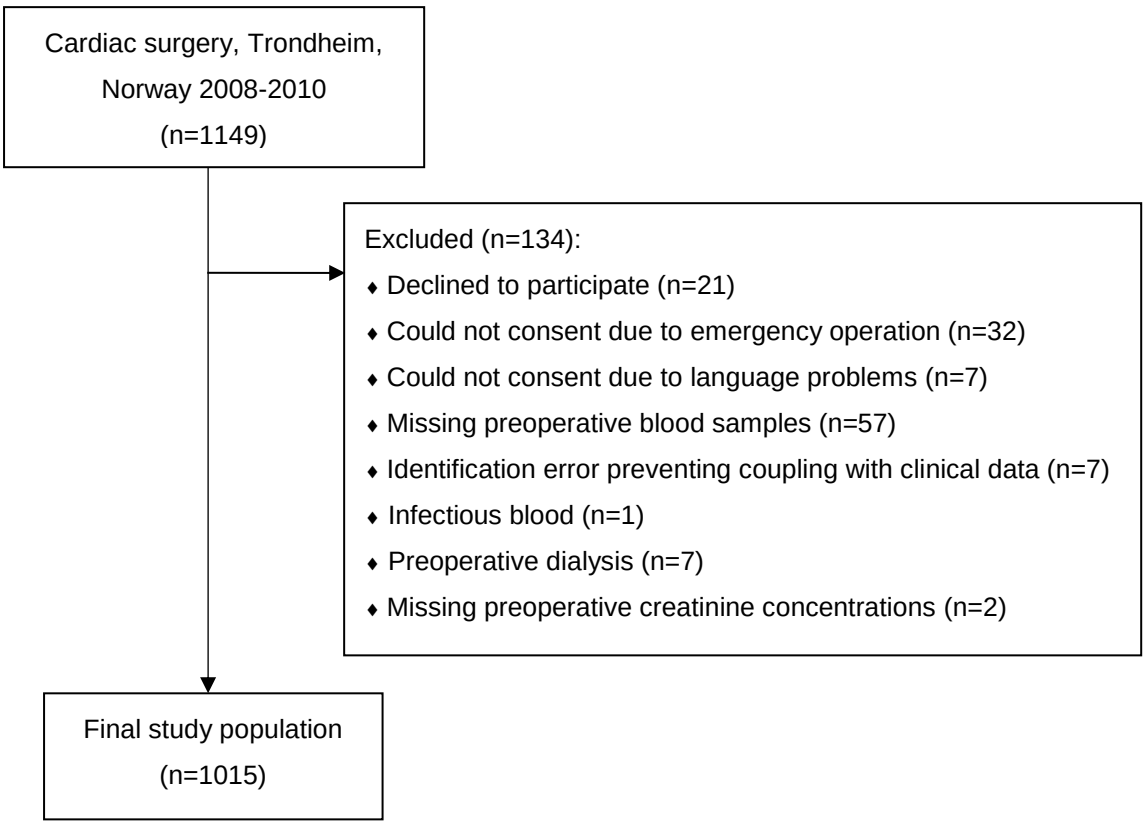

Figure S1: Patient inclusion and exclusion to the study

1.2 Measurements of ventricular ejection fraction

In patients registered into the Trondheim Heart Surgery Database, left ventricular ejection fraction (LVEF) has been determined by means of single-planed left ventricular (LV) angiography or noninvasively through two-dimensional echocardiography. Over time, echocardiography has become the main modality.

It has previously been shown that LVEF obtained by echocardiography tends to be lower compared to angiographic measurements.[1] A regression equation was derived based on patients undergoing cardiac surgery in Trondheim from 2000 through 2011 who had their LVEF measured by both methods. Estimated echocardiographic LVEFs were then calculated for those patients in the present study who underwent angiography only (n=194 (19%)). 566 patients (56%) underwent preoperative echocardiography. The remaining 255 patients (25%) had no clinical indication for LVEF measurement, and thus the LVEF was assumed to be >50%.

1.3 Selection of clinical adjustment variables

Table S1: Variables included in clinical risk models for acute kidney injury

CaSOS' CSA-AKI risk model[a]	Cleveland clinical risk score[b]	UK any-stage AKI risk calculator[c]	Novel model[d]
Age	Female gender	Age - < 60 years† - 60-74 years - ≥ 75 years	Age
BMI >30 kg/m^2	Congestive heart failure	Female gender	Female gender
Lipid-lowering treatment	Left ventricular ejection fraction <35%	BMI (kg m^{-2}) - <20.0 - 20-0-24.9 - 25.0-29.9† - 30.0-34.9 - ≥ 35.0	BMI >30 kg/m^2
Hypertension	Preoperative use of IABP	Smoking - Never smoked† - Ex-smoker - Current smoker	≥ 2 surgical procedures
Peripheral vascular disease	Chronic obstructive pulmonary disease (medically treated)	Dyspnoea - NYHA class I† - NYHA class II - NYHA class III - NYHA class IV	Preoperative creatinine
Chronic pulmonary disease	Insulin-dependent diabetes	Diabetes	
Haemoglobin concentration	Previous cardiac surgery	Peripheral vascular disease	
Preoperative creatinine - Below 100 µmol/l† - 100 to 140 µmol/l - Above 140 µmol/l	Emergency surgery	Hypertension	
Previous cardiac surgery	Surgery type - CABG only† - Valve only - CABG + Valve - Other cardiac surgeries	Haemoglobin (g/dl) - <10.0 - 12.0-11.9 - ≥ 12.0†	
Emergency surgery	Preoperative creatinine* - < 1.2 mg/dl† - 1.2-2.1 mg/dl - ≥ 2.1 mg/dl	GFR (mL/min per 1.73 m^2) - <30.0 - 30.0-59.9 - 60.0-89.9† - ≥ 90.0	
Surgery type - CABG and ASD† - AVR, AVR and CABG, non-ischaemic mitral valve surgery, aneurysm of ascending aorta - Dissection of ascending aorta, rupture of the ventricular septum - Miscellaneous		PCI prior to surgery	
		Triple vessel disease	
		Ejection fraction - Good (≥ 50%)† - Fair (30-49%) - Poor (<30%)	
		Operative priority - Elective† - Urgent - Emergency surgery	
		Surgery type - CABG only† - Valve only - CABG + Valve - Other/multiple cardiac surgeries	

Three previously described clinical models were validated in the present study cohort. This table provides an overview over the AKI predictors included in the published models, as well as the variables constituting the final clinical model used for adjustment of the biomarker analysis. ASD, atrium septum defect; AVR, aortic valve replacement; BMI, body mass index; CABG, coronary artery bypass grafting; CSA-AKI, cardiac surgery-associated acute kidney injury; IABP, intra-aortic balloon pump; GFR, glomerular filtration rate; NYHA, The New York Heart Association Functional Classification (I-IV); PCI, percutaneous coronary intervention.

[†]Defined as reference categories

*Categories correspond to <106.1 µmol/l, 106.1 µmol/l-185.64 µmol/l and >185.64 µmol/l. Conversion factor from mg/dl to µmol/l; x 88.4.

[a]**CaSOS' CSA-AKI risk model:** Berg KS, Stenseth R, Wahba A, et al.: How can we best predict acute kidney injury following cardiac surgery?: A prospective observational study. Eur J Anaesthesiol. 30:704-712, 2013.

[b]**Cleveland clinical risk score:** Thakar CV, Arrigain S, Worley S, et al.: A clinical score to predict acute renal failure after cardiac surgery. J Am Soc Nephrol. 16:162-168, 2005.

Due to incomplete registration of medical treatment for chronic obstructive lung disease, we included all patients registered with chronic obstructive lung disease. The risk score was originally developed for dialysis-dependent renal failure, but was presently applied on all patients with CSA-AKI.

[c]**UK any-stage AKI risk calculator**: Birnie K, Verheyden V, Pagano D, et al.: Predictive models for kidney disease: improving global outcomes (KDIGO) defined acute kidney injury in UK cardiac surgery. Crit Care. 18, 2014.

Information about PCI prior to surgery and triple vessel disease was not available, and were therefore excluded from the calculations.

[d]For a detailed description of novel model development, see the main article.

Model performance and model selection strategies

Model discrimination, i.e. the extent to which the model successfully separates between cases and non-cases, was evaluated by the area under the receiver-operating characteristic curve (AUC). The AUC may indicate that a model can have an excellent discriminative ability, however, it gives no information about calibration. Calibration refers to the agreement between observed and predicted outcomes and may be assessed by the Hosmer-Lemeshow test. A high p-value (>0.05) indicates appropriate calibration.

Log likelihood is an estimate of the probability of observing the data given the parameter estimates and the specified model. However, adding more terms to a model will usually improve the fit and thus lead to the acceptance of more complex models. The Akaike and Bayesian information criteria (AIC and BIC, respectively) are useful goodness-of-fit statistics to select the model in a set of candidate models giving the best balance between model fit and complexity. Both penalize for the number of estimated parameters, where BIC penalizes more for additional parameters and thus minimizes the risk of overfitting. The best model is generally the one that minimizes both AIC and BIC.

1.4 Assessing the incremental value of novel predictors

The increase in the area under the receiver operating characteristics curve (AUC), integrated discrimination improvement (IDI) and net reclassification improvement (NRI) provide complementary information and have been recommended as three parallel measures that form the "first line of assessment" in pre-screening of novel biomarkers.[2]

IDI is a measure of the separation in predicted probabilities between events and non-events. However, IDI depend on the incidence of the outcome of interest. Thus, the relative IDI (rIDI), defined as the increase in discrimination slopes divided by the slope of the old model, was calculated.[3] A rIDI ≥ the inverse number of variables in the baseline model indicates that the added predictors performed better than the average contribution of each clinical variable.

The NRI is derived by calculating the net proportion of events and non-events reclassified correctly with the new risk algorithm. Net correct reclassification for patients suffering CSA-AKI is calculated as the proportion of AKI cases in the test dataset who are correctly reclassified to a higher risk category minus the proportion of cases that are incorrectly reclassified to a lower risk category. Correspondingly, for the controls, the proportion of patient incorrectly being reclassified to higher risk categories are subtracted from the proportion being correctly reclassified to lower risk categories. The overall categorical NRI is then the sum of the net correct reclassification in cases and controls.

Use of categorical NRI should be based on risk thresholds which have clear clinical implications and are motivated on clinical grounds.[4] Pencina and colleagues have proposed that in situations with no established cut-offs, using the event rate as the default risk threshold may not be unreasonable.[5] Thus, we defined three categories of low, intermediate and high risk, corresponding to < 10%, 10-20% and > 20% predicted risk calculated from the clinical model, respectively. Additionally, we compared these results with cut-offs at < 25%, 25-50% and > 50% predicted risk, as applied by a previous study investigating the impact of preoperative cystatin C on AKI risk prediction.[6] Due to lack of clear risk thresholds for CSA-AKI, we also calculated the "continuous NRI" or NRI(>0). The continuous NRI has been recommended in situations where the primary focus is on the strength of the marker rather than model performance.[4] A continuous NRI >0.6, around 0.4 and <0.2 are considered strong, intermediate and weak, respectively.[2]

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Supplementary Material 2: Supplementary results

2.1 Correlations among variables

Table S2: Pearson's correlation coefficients amongst a) biomarkers and b) different indicators of renal function

a)

	CRP	TCC	Lactoferrin	Neopterin	Cystatin C	NT-proBNP
C-reactive protein (CRP)	1.00					
Terminal complement complex (TCC)	0.03	1.00				
Lactoferrin	-0.05	0.03	1.00			
Neopterin	0.32***	0.05	0.01	1.00		
Cystatin C	0.14***	0.09**	0.00	0.40***	1.00	
NT-proBNP	0.14***	0.01	-0.03	0.27***	0.30***	1.00

b)

	Creatinine	Creatinine clearance	Cystatin C	eGFR-cr	eGFR-cr+cys
Creatinine	1.00				
Creatinine clearance[a]	-0.51***	1.00			
Cystatin C	0.48***	-0.32***	1.00		
eGFR-cr[b]	-0.79***	0.80***	-0.47***	1.00	
eGFR-cr+cys[c]	-0.63***	0.64***	-0.85***	0.81***	1.00

*p=0.05, **p<0.01, ***p<0.001.

[a]Creatinine clearance calculated from the Cockcroft-Gault equation.[1]

[b]eGFR-cr: Estimated glomerular filtration rate calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (2009).[2]

[c]eGFR-cr+cys: Estimated glomerular filtration rate calculated with CKD-EPI creatinine-cystatin C single equation (2012): $135\times\min(Scr/\kappa, 1)^{\alpha}\times\max(Scr/\kappa, 1)^{-0.601}\times\min(Scys/0.8, 1)^{-0.375}\times\max(Scys/0.8, 1)^{-0.711}\times0.995^{Age}$ [×0.969 if female][×1.08 if black], where Scr is serum creatinine, Scys is serum cystatin C, κ is 0.7 for females and 0.9 for males, α is −0.248 for females and −0.207 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.[3]

2.2 Alternative multivariate models

Table S3: Comparison of AKI risk model performance in patients undergoing cardiac surgery

	CaSOS' CSA-AKI risk model		**Cleveland clinical risk score**		**UK any stage AKI calculator**	**Novel model**
	Calculated predicted probability (%)	Predictor variables	Calculated risk score (range 0-17)	Predictor variables	Calculated predicted probability (%)	Predictor variables
a) Model statistics						
Degrees of freedom	2	13	2	12	2	6
Log likelihood	-274.7	-259.7	-284.2	-276.8	-275.8	-264.3
AIC	553.5	545.3	572.5	577.6	555.6	540.7
BIC	563.3	609.2	582.3	636.5	565.4	570.2
AUC	0.802	0.816	0.745	0.772	0.778	0.805
(95% CI)	(0.759-0.845)	(0.775-0.858)	(0.694-0.796)	(0.721-0.823)	(0.729-0.827)	(0.763-0.847)
Hosmer-Lemeshow test	0.004	0.02	0.40	0.56	0.11	0.46
b) Biomarker evaluation	Level of significance					
Lactoferrin[a]	0.15	0.10	0.12	0.08	0.06	**0.05**
NT-proBNP[b]	**<0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Neopterin[a]	0.12	**0.03**	**0.02**	**0.004**	0.07	**0.04**
C-reactive protein[a]	0.43	0.80	0.41	0.57	0.75	0.38
Cystatin C[a]	0.11	0.12	**0.03**	**0.04**	0.09	0.13

a) Summary of the performance of the clinical risk models and model comparison statistics. b) Summary of the multimarker analysis for the biomarkers when adjusted with each of the clinical models. Significant biomarkers are shown in bold.

Clinical models were compared in 1,004 patients with complete data on all variables for all models. Comparison of models including biomarkers was performed on n=996, after exclusion of 8 patients with incomplete biomarker data. The CaSOS' CSA-AKI model, Cleveland clinical risk score and UK any stage AKI calculator were tested by adding the calculated risk score as a continuous variable in a multivariate model with the biomarkers. Additionally, we tested the alternative strategy using the variables described by the CaSOS' CSA-AKI model and Cleveland clinical risk score in new model versions with recalculated model coefficients.

AIC, Akaike information criterion; AUC, area under the receiver-operating characteristic curve; BIC, Bayesian information criterion; HL test, Hosmer-Lemeshow test; NT-proBNP, N-terminal pro-brain natriuretic peptide.

[a]Natural log-transformed. [b]Dichotomized with a cut-off >125 pg/ml

2.3 Alternative indicators of renal function

Table S4: Comparison of novel AKI risk prediction models with different indicators of renal function

Predictor variable	**Odd ratios with level of significance**			
	Creatinine	Creatinine clearance	eGFR-cr	eGFR-cr+cys
Female gender	0.64	.54*	.55*	.53*
Age (per 5 years)	1.12	1.04	1.05	1.06
Body mass index >30 kg/m^2	2.68***	3.21***	2.58***	2.48***
Multiple surgical procedures	3.46***	3.42***	3.33***	3.36***
NT-proBNP >125 pg/ml	2.90***	2.90***	2.87***	2.82***
Neopterin[a]	2.87***	3.33***	2.62**	2.59**
Lactoferrin[a]	.67*	.68*	.69*	.68*
Serum creatinine (per 10 µmol/l)	1.08	-	-	-
Creatinine clearance[b] (ml/min)	-	0.99	-	-
eGFR-cr[c] (ml/min per 1.73 m^2)	-	-	.98*	-
eGFR-cr+cys[d] (ml/min per 1.73 m^2)	-	-	-	.91**
Model comparison estimates				
Log likelihood	-251.8	-252.9	-250.8	-250.0
Akaikes information criterion	521.6	523.9	519.7	518.0
Bayes information criterion	565.9	568.1	563.9	562.2

*0.05, **<0.01, ***<0.001. NT-proBNP, N-terminal pro-brain natriuretic peptide.

[a]Natural log-transformed.

[b]Calculated from the Cockcroft-Gault equation.[1]

[c]eGFR-cr: Estimated glomerular filtration rate calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (2009).[2]

[d]eGFR-cr+cys: Estimated glomerular filtration rate calculated with the CKD-EPI creatinine-cystatin C equation (2012).[3]

As shown by the model comparison statistics, there were only small differences. Parameter estimates for novel biomarkers were consistent independently of how renal function was modelled. However, the model using estimated GFR based on both creatinine and cystatin C showed somewhat better performance and was thus used as the final model for clinical adjustment of the multivariate biomarker analysis.

2.4 The incremental value of added biomarkers

The AUC difference indicated a marginally significant improved discrimination (p=0.05). However, AUC comparison is considered a conservative measure, and it is considered especially difficult to improve baseline models with large AUCs.[4] Nevertheless, there was a significant improvement in the ability to separate between AKI events and non-events (IDI=0.06 (0.03-0.08), p<0.001). The calculated relative IDI of 0.37 indicates that the incremental contribution by the biomarkers was higher than the average contribution from each clinical variable (1/5=0.20).

The continuous NRI was 0.55 (0.34-0.75). The categorical NRI showed a net reclassification of 12% (p=0.05), where a net of 10% of AKI cases were correctly reclassified to higher risk categories (Table 4). Inclusion of biomarker data had the largest impact in the intermediate risk group (predicted risk 10-20%, n=175), where a net of 27% and 29% of AKI and non-AKI cases were reclassified into higher and lower risk categories, respectively. The overall NRI in the intermediate group was therefore 56%.

A similar analysis for risk categories at cut-offs 25% and 50% resulted in a NRI of 23% (p<0.001). The estimates were of greater magnitudes, but fewer patients reached the higher risk categories. A net of 24% of AKI cases were correctly reclassified to higher risk categories (Table 4). Inclusion of biomarker data had the largest impact in the intermediate risk group (predicted risk 25-50%, n=68)). From 23 AKI cases, a net of 9 patients were correctly reclassified to a higher risk group (12-3, 39%), whereas a net of 9 from 45 non-AKI cases were correctly reclassified to a lower risk category (15-4, 20%). Thus, there was an overall NRI of 59% in the intermediate risk group using these alternative cut-offs.

Table S5: Comparison of risk classification for AKI based on clinical variables only and combined biomarkers and clinical variables.

A) Main analysis

		Risk classification with biomarkers and clinical variables			
		< 10% risk	10-20% risk	> 20% risk	Total
	Risk classification with clinical variables only				
AKI cases	< 10% risk	20	**8**	**2**	*30*
	10-20% risk	*5*	6	**11**	*22*
	> 20% risk	*2*	*4*	42	*48*
	Total	27	18	55	100
Non-AKI cases	< 10% risk	614	*52*	*4*	670
	10-20% risk	**71**	56	*26*	153
	> 20% risk	**5**	**20**	59	84

	Total	690	128	89	907

B) Sensitivity analysis

		Risk classification with biomarkers and clinical variables			
		< 25% risk	25-50% risk	> 50% risk	Total
	Risk classification with clinical variables only				
AKI cases	< 25% risk	49	**18**	**0**	67
	25-50% risk	*3*	8	**12**	23
	> 50% risk	*0*	*3*	7	10
	Total	52	29	19	100
Non-AKI cases	< 25% risk	827	*24*	*0*	851
	25-50% risk	**15**	26	*4*	45
	> 50% risk	**0**	**3**	8	11
	Total	842	53	12	907

Reclassification tables when using cut-offs at A) 10% and 20% predicted AKI risk; and B) 25% and 50% predicted AKI risk. Values represent number of patients (n). Correct reclassification is indicated with bold; incorrect reclassification in italics. AKI, acute kidney injury.

References

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