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Duration of cardiopulmonary bypass is an important confounder when using biomarkers for early diagnosis of acute kidney injury in cardiac surgical patients

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Abstract

Objectives: Acute kidney injury is an important complication in patients undergoing cardiac surgery. Recently, several biomarkers to facilitate early detection of acute kidney injury have been proposed, among them Neutrophil-gelatinase-associated-lipocalin, Kidney-injury-molecule-1, and L-Fatty-acid-binding-protein. The expression of these markers is increased by ischemia-reperfusion injury and may thus be related to the duration of cardiopulmonary bypass (CPB). The present study was designed to determine the effects of CPB-duration on the postoperative course of acute kidney injury biomarkers in comparison with patients developing acute kidney injury according to the classical definition of a decrease in creatinine clearance in a cohort of cardiac surgery patients.

Methods: Prospectively sampled data from 136 consecutive patients were analyzed retrospectively. Plasma and urine for determination of biomarkers and creatinine were collected at predefined time points before, immediately after, and up to three days after surgery. The median duration of CPB was 118 min. 29 patients developed acute kidney injury as defined by the acute kidney injury network creatinine criteria. The patients without acute kidney injury were grouped into a "CPB–short" (CPB < 118min; n = 51) and a "CPB–long" (CPB ≥ 118min; n = 56) group.

Results: Preoperative plasma Neutrophil-gelatinase-associated-lipocalin and urinary L-Fatty–acid-binding-protein levels were higher in the acute kidney injury than in the CPB-short group. Early postoperative levels of plasmatic and urinary Neutrophil-gelatinase-associated-lipocalin and urinary L-Fatty-acid-binding-protein increased significantly after CPB in all groups and were either higher or not different in comparison between the CPB-long and the acute kidney injury group in the immediate postoperative period.

Conclusion: Duration of CPB is a relevant factor for the expression of several biomarkers of renal tubular injury presumed to facilitate early detection of acute kidney injury after cardiac surgery. This questions the usefulness of the markers used in this study for early detection of renal dysfunction and prediction of a further decrease in glomerular filtration rate after cardiac surgery and suggest that CPB-time has to be taken into account when defining cut-off levels for acute kidney injury biomarkers in this setting.

Key words: Renal biomarkers, acute kidney injury, cardiac surgery, cardiopulmonary bypass

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Introduction

Cardiac-surgery associated acute kidney injury (CSA-AKI) remains an important and frequent complication in patients undergoing cardiac surgery and is associated with a poor short- and long-term prognosis [1]. The incidence for CSA-AKI according to Acute Kidney Injury Network criteria (AKIN) varies between 3% and 50% [1]. CSA-AKI requiring temporary renal replacement therapy (RRT) occurs in 5% to 20% [2] of these patients and is associated with a high mortality rate [1].

Early detection of AKI is deemed important to develop therapeutic concepts to treat or at least ameliorate a renal insult. Within the last years various biomarkers reflective of ischemic tubular injury have been developed to accomplish this task, among them Neutrophil-gelatinase-associated lipocalin (NGAL) [3,5], Kidney-injury molecule -1 (KIM-1) [3], and L-Fatty-acid-binding-protein (L-FABP) [3,5].

However, most of these novel AKI markers have been derived from animal experiments inducing *renal* ischemia-reperfusion injury [3], and since cardiac surgery with cardiopulmonary bypass (CPB) may be regarded as a prototype clinical scenario of *systemic* ischema-reperfusion injury [6,7], the duration of CPB may influence the postoperative course of these peptides.

In line with this assumption, Wagener and coworkers have observed that the postoperative levels of urinary NGAL were related to the duration of cardiopulmonary bypass (CPB) in a cohort of 426 patients undergoing cardiac surgery [6] and observed, that the predictive capacity of urinary NGAL for AKI was comparable to the predictive ability of CPB-duration. These findings have important implications since prolonged CPB is an important risk factor for AKI [5] and may hence be a relevant confounder for the interpretation of postoperative AKI-biomarkers. No comparative data are available on the effects of CPB-duration on the postoperative course of other putative markers of AKI in patients with preserved postoperative renal function in relation to the duration of CPB in comparison with patients developing CSA-AKI.

This study was thus designed to explore the effects of CPB-duration on AKI-biomarkers in a cohort of cardiac surgery patients with preserved postoperative renal function in comparison with patients presenting with overt CSA-AKI according to the Acute Kidney Injury Network AKIN criteria [8].

Material and methods

The present analysis is a substudy of an observational trial on the relationship between preoperative cerebral oxygen saturation and postoperative organ dysfunction as detailed elsewhere [9]. The study was approved by the local ethics committee (Ethikkommission der Universität zu Lübeck, Lübeck, Germany; reference number: 07-146; amendment 2) and all patients provided informed consent. Prospectively sampled data from 136 consecutive adult patients that had undergone cardiac surgery at our institution from April, 1st 2009 to May, 31st 2009 were used for the present retrospective analysis. Exclusion criteria were age less than 18 years, preoperative endstage chronic kidney disease, and off-pump cardiac surgery.

Patient demographics, surgical core data including CPB duration, postoperative morbidity (including the need for renal replacement therapy), and mortality were derived from the cardiac surgery quality register. Estimated preoperative creatinine clearance was calculated according to the Modifications-of-Diet-in-Renal-Disease equation (MDRD) [10].

Plasma samples for determination of creatinine and NGAL were determined at the following time points: (t1) before induction of anesthesia; (t2) at the end of surgical procedure; and (t3, t4, t5) on the morning of the first to third postoperative day. Urine samples for determination of creatinine, NGAL, KIM-1, and L-FABP were determined at the following time points: (t1) before induction

of anesthesia; (t2) at the end of surgical procedure; and 6h (t3), 12h (t4), and 24h (t5) after surgery. The samples were spun with 2.500 rounds per minute for 10 minutes and the supernatants were stored at –80°C for further analysis. The excretion of all urinary markers were adjusted to creatinine excretion.

Definition of AKI

AKI was defined as stage I (serum creatinine increase of 50% to 100% (1.5- to twofold) or ≥ 0.3 mg/dl), stage II (serum creatinine increase of >100% to 200% (more than two- to threefold)) and stage III (serum creatinine increase of >200% (more than threefold) or new need for renal replacement therapy)) according to Acute Kidney Injury Network AKIN criteria [8].

Analysis of specific biomarkers

Quantitative analyses of specific biomarkers were performed by using commercially available Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) kits in accordance to the manufacturer's instructions.

Plasma and urinary NGAL concentrations were determined with a sandwich ELISA kit (Antibodyshop, Gentofte, Denmark). The limit of detection for this assay is 0.02 ng/ml. The intra- and interassay coefficient of variation is 7.7% and 9.75%, respectively.

The urinary KIM-1 concentration was analyzed with a sandwich ELISA (R&D Systems, Minneapolis, MN, USA). The lower limit of detection was 0.05 ng/ml, and the intra- and interassay coefficient of variation was 9.4% and 12.9%, respectively.

L-FABP levels were quantified by a sandwich ELISA (CMIC Co. Ltd, Tokyo, Japan). The range of this kit is between 4 and 400 ng/ml.

Anesthesia, surgery, and cardiopulmonary bypass management

All patients were monitored with invasive arterial and central blood pressure monitoring. If deemed necessary by the attending anesthetist or in patients undergoing complex cardiac surgery, semicontinuous cardiac output, continuous mixed venous oxygen saturation, and pulmonary artery pressures were determined by a pulmonary artery catheter. Transesophageal echocardiography was performed in all valve surgery cases.

Cerebral oxygen saturation (ScO_2) was determined in all patients with an INVOS® 5100 monitor (Somanetics, Troy, USA) as described previously [9]. Oxygen supplemented baseline ScO_2 values were used for titrating perioperative perfusion.

Anesthesia was induced with sufentanil (0.5-1 μg/kg BW) and etomidate (0.2-0.4 mg/kg BW) and maintained at (0.8-1.0 minimal alveolar concentration) of sevoflurane and remifentanyl (0.2-0.3 μg kg/min) with the goal of early postoperative extubation. Muscle relaxation was achieved with rocuroniumbromide (0.6 mg/kg BW). During CPB, propofol in a dose of 4-5 mg/kg BW/h was applied since at that time volatile anesthetics could not be given via the CPB circuit. All patients were ventilated in a volume controlled mode; with a tidal volume of 6 ml/kg BW and respiratory rate adjusted to achieve normocapnia.

Surgical procedures were performed with CPB in moderate or deep hypothermia. Cardioplegic arrest was achieved by blood cardioplegia and repeated every 20 minutes. In coronary-artery bypass grafting (CABG) cases, single cross-clamp was used. Non-pulsatile perfusion was performed during CPB. Pump flow, oxygen flow, and MAP were adjusted to maintain ScO2 levels within the preoperative range and at least higher than 50% absolute.

Upon admission to the ICU, the continuous infusions of propofol and remifentanyl were stopped if the patient was hemodynam-

ically stable and normothermic (core temperature >36°C). Hemodynamic stability was defined as a mean arterial blood pressure (MAP) of 60 to 90 mmHg, heart rate (HR) between 60 to 90 bpm, a central venous pressure (CVP) between 10 to 15 mmHg and central venous oxygen saturation (ScvO2) greater than 70%. In patients monitored with pulmonary artery catheter (PAC), hemodynamic therapy was titrated to achieve cardiac index > 2.2 L/min/m2 and mixed venous oxygen saturation (SvO2) greater than 65 % [11]. Fluid therapy was performed with balanced crystalloid (Sterofundin ISO 1/1; BBraun; Melsungen, Germany) and colloids (Volulyte®, Fresenius, Germany; Gelafundin®, BBraun, Melsungen, Germany) fluids, as suggested by a recent guideline [11].

Statistical analysis

For analysis, patients were subdivided into three groups: AKI (AKI-group), short duration of CPB and no AKI (CPB-short), long duration of CPB and no AKI (CPB-long). T-tests as well as Mann-Whitney U-test, where appropriate, were used to investigate for differences in demographics. The classification ability of the studied biomarkers was also analysed and presented with receiver operating characteristics (ROC) curves.

All analyses were performed with R version 2.15.2 [R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, http://www.R-project.org/]. If not stated otherwise, data are given as mean ± standard deviation (for normaly distributed variables) or median (1st and 3rd quantile) for non-parametric distributions. A $p < 0.05$ indicates statistical significance.

Results

Median duration of cardiopulmonary bypass was 118 min. Twenty nine patients (21.3%) developed renal dysfunction according to the AKI network criteria. The incidence of AKI grade 1 to 3 was 4.4% (n = 6), 11.7% (n = 16), and 5.1% (n = 7), respectively. 107 patients did not fulfil the AKI criteria: n = 51 with a CPB-duration < 118 min (CPB – short) and n = 56 with a CPB-duration ≥ 118 minutes (CPB-long).

Demographics, surgical core data and clinical outcomes

Demographics and surgical core data in the different groups are presented in table 1. Patients in the AKI group were older and scored higher in relation to NYHA-categories as well as in logistic- and additive Euroscore. Duration of surgery, CPB-duration, and aortic cross-clamp time were longer in the AKI than in the CPB-short but not different from the CPB-long group. Clinical outcomes in the different groups are presented in table 2.

Specific biomarkers for CSA-AKI

The time course of plasma and urinary levels of renal biomarkers in the different groups are presented in table 3 and 4, showing that all biomarkers increased significantly after surgery in comparison with baseline values.

Plasma NGAL levels peaked immediately postoperatively (t2) and no differences were observed between patients in the CPB-long and the AKI group immediately postoperatively (t2) and on postoperative day one (t3). Thereafter, and paralleling the evolution of plasma creatinine levels, plasma NGAL increased in the AKI group and were significantly higher at t4 and t5 in comparison with the CPB-short and the CPB-long group. Urine NGAL and L-FABP levels immediately postoperatively (t2) were significantly higher in the CPB-long group than in the AKI-group. Comparably, urinary KIM-I levels immediately postoperatively (t2) were also significantly higher in the CPB-long group than in the AKI-group. No further differences in urine NGAL, L-FABP, and KIM-I levels were observed between patients in the CPB-long and the AKI group within the first 24h.

ROC analyses

The results of the ROC analyses revealed a limited classification ability of the biomarker levels at (t1, t2 and t3) for the development of CSA-AKI. The resulting area under the curve for any of these biomarkers at any time point did not exceed the value of 0.73, thus offering limited information for the classification of patients that developed CSA-AKI in the further course (table 5)

Univariate and multivariate analysis

A univariate binary logistic regression analysis (table 6) including duration of surgery, duration of cardiopulmonary bypass, aortic cross-clamp time, age, height, weight, sex, surgical priority, left ventricular ejection fraction, Logistic EuroSCORE, delta creatinine (t2-t1), eGFR (t1), urinary L-FABP (t1,t2), plasma NGAL (t1,t2), and urinary NGAL (t1-t2) revealed that the Logistic EuroSCORE, plasma NGAL (t1), L-FABP (t1), and delta creatinine (t1,t2) were statistically significant predictors for the development of postoperative CSA-AKI. A multivariate binary logistic regression analysis (table 6) revaled that only age was a consistent independent predictor of CSA-AKI.

Discussion

Despite many years of research, AKI remains an important and life threatening complication in patients undergoing cardiac surgery, and with respect to high incidence of this complication in this specific population has even got a sub-designation: CSA-AKI [5]. Comparable to other clinical settings [5,12], early detection of this complication is deemed pivotal to develop strategies to ameliorate and modify kidney injury at the earliest stages to prevent a further deterioration of renal function and the accompanying consequences [7].

Based on findings during experimental renal injury, a number of proteins specifically upregulated following an ischemic insult were identified [4] and subsequently studied in various clinical scenarios as putative biomarkers for the early detection of AKI [3,4,5,7,13]; among them NGAL, KIM-1, and L-FABP. With respect to the high incidence of AKI in patients undergoing cardiac surgery, and the well defined insult of surgery and CPB, the diagnostic capacity of AKI-biomarkers for the early detection of AKI has been repeatedly analyzed in this population of patients. And despite highly variable [1,5,13] findings, recent reviews came to the conclusion, that urine and plasma NGAL [13] and urine KIM-1 levels (when AKI was defined conventionally as an increase in serum creatinine) may detect AKI at earlier stages than creatinine and that these markers may thus be helpful for the early detection of AKI in various clinical settings [14].

In contrast to these findings, mostly derived from small studies in mixed populations of critically ill patients, Wagener and coworkers presented several analyses questioning the usefulness of NGAL, the currently most frequently analyzed AKI-biomarker, for the early detection of AKI in patients undergoing cardiac surgery: This group [6] showed a comparable (and inacceptably low predictive capacity) of urinary NGAL levels in a cohort of 426 adult cardiac surgery patients that was comparable to the predictive capacity of CPB-duration. A secondary analysis revealed that the ability of urinary NGAL to provide early and accurate identification of evolving AKI was significantly influenced by baseline eGFR and that the clinical usefulness of this marker was restricted to patients with normal baseline renal function [15]. Comparably, Cai and colleagues showed that urinary NGAL levels were positively correlated to extracorporeal circulation time in 59 patients undergoing cardiac surgery [16].

Seitz and coworkers analyzed the predictive capacity of postoperative urinary NGAL and plasma Cystatin C levels in pediatric patients undergoing cardiac surgery [1]. In this study, Cystatin C 2h after surgery was a bet-

ter predictor of AKI, despite the AUC ranged between 0.71 and 0.74. No correlation was observed between urinary NGAL levels and the subsequent course of creatine; however, significant correlations were observed between urinary NGAL levels, duration of CPB and aortic crossclamp time, postoperative plasma lactate levels, and duration of mechanical ventilation in these patients.

The largest study (1219 adults undergoing cardiac surgery) on the predictive capacity of urinary and plasmatic NGAL and Interleukin-18 revealed that the area-under-the-curve (AUC) of a clinical model for predicting AKI was improved by adding Interleukin-18 or urinary NGAL [17]. However, the AUC and the net-reclassification-improvement (NRI) of these markers were far from being convincing with AUC's between 0.73 to 0.76 and the NRI of NGAL even reaching statistical significance ($p = 0.05$).

The results of the present study clearly show, that the plasma and urinary levels of the biomarkers NGAL, KIM-1, and L-FABP were significantly increased in patients with a prolonged duration of CPB in comparison with a short time on extracorporal circulation and failed to discriminate between patients with preserved postoperative renal function in comparison with patients presenting with overt CSA-AKI according to AKIN criteria. This questions the usefulness of fixed cut-off levels of AKI biomarkers (if derived from heterogeneous populations of critically ill) and suggest that – at least in cardiac surgical patients – any cut-off level initiating a therapeutic response should be adjusted according to the duration of CPB.

Our results for NGAL are in partial contrast to older studies analyzing the predictive capacity of NGAL either in pediatric [1,7] or adult [5,6] cardiac surgical patients but clearly supported by the most recent investigations describe above. In addition to the use as biomarker for the detection of renal injury, several other publications point to the fact that NGAL does not only serve as markers for *renal,* but also for *systemic* ischemia-reperfusion [17,18]. Valette et al. analyzed the predictive capacity of NGAL for the early detection of contrast-induced AKI in a heterogeneous population of critically ill patients and observed that postinterventional plasma levels of NGAL failed to predict AKI in this cohort of 100 patients but were significantly higher in patients with sepsis [19]. Comparably, Maisel and coworkers showed that the plasma NGAL was increased in patients with heart failure and that high NGAL plasma levels – especially if combined with increased B-type natriuretic peptide (BNP) plasma concentrations – were predictive for a combined endpoint of readmission for decompensated heart failure and mortality [20]. It is of note that plasma creatinine as well as eGFR were not predictive in this setting.

Few data are available on the usefulness of L-FABP in the setting of cardiac surgery. In a cohort of 85 patients Matsui and coworkers [5] showed that preoperative L-FABP, NGAL and Albumin levels were significantly higher in patients with AKI in comparison with patients not developing AKI group and that urinary L-FABP – either preoperatively and 6h after surgery – was an early predictor for AKI in patients undergoing cardiac surgery. Duration of CPB was unaccounted in this study.

Comparably to L-FABP, sparse data are available on the usefulness of KIM-1 for predicting AKI in cardiac surgical patients. Koyner and coworkers showed in 123 cardiac surgery patiens that preoperative KIM-1 levels were able to predict the future development of stage 1 and stage 3 AKI. However, postoperative levels of this peptide did not further improve diagnostic accuracy [21].

Limitations and controversies

This study has limitations and may raise controversies.

1. Due to the observational design, the population of this study is highly heterogeneous. However, under "real life" conditions, one will be confronted with comparable heterogeneity.

2. With respect to the observational nature of this study we did not perform an a priori power analysis to determine if the sample size is adequate. Thus one has to keep in mind that the data were derived from a relatively small sample and by retrospective analysis. Consequently these findings need to be reproduced prospectively and in an independent and larger population.
3. In a recent statement, the Acute-Dialysis-Quality-Initiative has suggested to expand the current functional definition of AKI that was based on the determination of changes in creatinine clearance and urine flow by adding the degree of kidney tissue damage determined by kidney-specific biomarkers [22]. Following this concept, kidney damage may be defined either by tissue damage and/or loss of function; thereby covering a spectrum from isolated tissue damage without functional deterioration (i.e. only an increase in kidney-specific biomarkers) to tissue damage with accompanying loss of function (i.e. increased biomarker expression plus decrease in glomerular filtration rate and/or urine flow) [22,23]. However, the ADQI also clearly states that – at present – a precise definition of AKI based on biomarkers is not possible until the factors leading to increased biomarkers expression and the respective associations with outcome have been clearly elucidated. The present study was not powered to perform meaningful analyses of an association between the postoperative increase in AKI-biomarkers and long-term outcome. However, taking into account "systemic contamination" (the fact that renal biomarkers are not only reflective of renal, but also of systemic malperfusion [19,21]) such analyses will need a much higher number of patients than enrolled for the present analysis.
4. Astonishingly, neither duration of surgery nor duration of CPB were associated with functional CSA-AKI in the present study. This is in clear contrast to several other studies [5,6] but may – at least in part – be related to strict optimization of blood flow and perfusion pressure during CPB according to physiological goals including ScO2 as a measure of the adequacy of tissue perfusion [24].
5. Very recently, the product of the biomarkers Insulin-like growth factor binding protein 7 (IGFBP7) and Tissue-inhibitor-of-matrixmetalloproteinase I (TIMP-1) has been shown to have superior predictive capacity for AKI in a huge cohort of critically ill patients in comparison with several other previously described biomarkers, including those analyzed in the present study [25]. No data are available on the effects of CPB on the expression of these new markers (that were not available for us during the study period) and it thus remains unclear, wether IGFBP7 and TIMP-1 are less influenced by CPB duration than other AKI biomarkers.

Taken together, the present data clearly show that duration of CPB is a relevant factor for the expression of several biomarkers presumed to serve as early markers for renal dysfunction after cardiac surgery. The fact that patients with prolonged CPB but preserved renal function may have significantly higher postoperative biomarker levels than patients presenting with a decrease in renal function does of course not question that postoperative AKI-biomarker levels may have implications for long term prognosis but clearly questions the usefulness of the tested peptides for early detection of renal dysfunction and predicting a further decrease in glomerular filtration rate after cardiac surgery. The association between increased AKI-biomarker levels and long-term outcome independent from renal function changes (i.e. a decrease in creatinine clearance) needs to be tested in future and large scale trials.

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Table 1: Demographics, preoperative and surgical-related data

	Patients without CSA-AKI CPB-short (n=51)	Patients without CSA-AKI CPB-long (n=56)	Patients with CSA-AKI (n=29)	Significance *p-value*
Demographic data				
Sex Female Male	 n=12 (24%) n=39 (76%)	 n=14 (25%) n=42 (75%)	 n=10 (34%) n=19 (66%)	n.s.
Age (years)	63.7 ± 11.2	67.9 ± 10.8	71.8 ± 10.2	<0.05 #
Pre-operative data				
Mean cerebral oxygen saturation	70 ± 7.2	66.8 ± 6.7	65.9 ± 10	n.s.
Prev. cardiac surgery	n=4 (8%)	n=8 (14%)	n=9 (31%)	<0.05 #
Previous MI	n=18 (35%)	n=14 (25%)	n=11 (37%)	n.s.
ASA	3.0 ± 0.52	3.0 ± 0.44	3.07 ± 0.58	n.s.
Additive EuroScore	4.2 ± 2.7	6.2 ± 2.8	7.4 ± 3.8	<0.05 #*
Logistic EuroScore II	0.06 ± 0.09	0.11 ± 0.1	0.14 ± 0.13	<0.05 #
NYHA I-II	n=37 (73%)	n=29 (52%)	n=11 (38%)	<0.05 #
NYHA III-IV	n=14 (27%)	n=27 (48%)	n=18 (62%)	<0.05 #
LVEF 1 EF < 30% 2 EF 30-50 % 3 EF > 50%	 n=3 (6%) n=16 (31%) n=32 (63%)	 n=1 (2%) n=11(19%) n=44 (79%)	 n=1 (3.5%) n=8 (27.5%) n=20 (69%)	n.s.
eGFR § (ml/min/1.73m2)	84.2 ± 20.8	78.9 ± 24.3	71.9 ± 28.8	n.s.
Creatinine	84 ± 18.5	89.6 ± 23.6	97.0 ± 31.3	n.s.
History				
Carotis stenosis	n=2 (4%)	n=4 (7%)	n=3 (10%)	n.s.
Diabetes mellitus	n=14 (27%)	n=9 (7%)	n=11 (38%)	n.s.
COPD	n=6 (12%)	n=11(20%)	n=7 (24%)	n.s.
Atrial fibrillation	n=6 (12%)	n=15 (27%)	n=5 (17%)	<0.05 *
Arterial hypertension	n=39 (76%)	n=42 (75%)	n=22 (75%)	n.s.
Hyperlipidaemia	n=36 (70%)	n=32 (57%)	n=22 (75%)	n.s.
Type of surgery				
CABG CABG+Valve Valve Other	n=38 (73%) n=1 (2%) n=11 (23%) n=1 (2%)	n=9 (17%) n=25 (45%) n=19 (34%) n=3 (4%)	n=18 (62%) n=6 (20%) n=5 (18%)	n.s.
Surgical-related data				
Duration surgery (min)	227 ± 48.8	n=313 ± 95	302.28 ± 113.7	<0.05 #*
Duration CPB (min)	91.1 ± 17.2	166.4 ± 49	143.17 ± 69.9	<0.05 #*
Aortic cross-clamp time (min)	70.5 ± 17.4	129.3 ± 44.9	106.5 ± 49.6	<0.05 #*

For normally distributed continuous variables data are expressed as mean ± SD. Not-normally distributed data are given as mean (interquartile range), categorical data are given as n (%). Cerebral oxygen saturation levels of both hemispheres were averaged for analysis. Other operations included combined valve surgery, ascending aortic surgery and right atrial thrombectomy. Abbreviations: EuroSCORE: European System for Cardiac Risk Evaluation; COPD: Chronic obstructive pulmonary disease; ASA: American Society of Anesthesiology grading; CABG: coronary artery bypass grafting; CPB: cardiopulmonary bypas; eGFR: estimated glomerular filtration rate; LVEF: left ventricular ejection fraction; MI: myocardial infarction; NYHA: New York Heart Association grading. #: p<0.05 CPB-short versus AKI. *: p<0.05 CPB-short versus CPB-long. §: p<0.05 CPB-long versus AKI.

Table 2: Clincal course and complications

	Patients without CSA-AKI CPB-short (n=51)	**Patients without CSA-AKI CPB-long (n=56)**	**Patients with CSA-AKI (n=29)**	**Significance *p-value***
Clinical course				
Postoperative ventilation time, h	5 (4-7)	5 (5-9.25)	11(7-77)	<0.05 # * §
Duration in ICU, h	22 (18.5-24)	22 (19-61)	88(21-189)	<0.05 # *
Duration in IMC, h	23 (20-43)	35 (20-60.8)	19 (0-48)	<0.05 * §
Duration in HDU, h	51 (41-89)	98 (43.5-134.8)	139 (74-232)	<0.05 # * §
MAC Score (n)				
0	51(100%)	50 (89%)	15 (51.7%)	<0.05 § * #
1	no	6 (10.7%)	3 (10.3%)	n.s.
2	no	no	9 (31%)	
3	no	no	2 (6.8%)	
4	no	no	no	
Complications				
Re-admission on ICU	no	3 (5.3%)	no	
Need for CPR	no	5 (8.9%)	2 (6.8%)	n.s.
Reintubation	no	5 (8.9%)	9 (31%)	<0.05 §
Tracheostomy	no	1 (1.8%)	2 (6.9%)	n.s.
Reoperation	9 (17.6%)	9 (16%)	26 (89.6%)	<0.05 §
Status after 30 days				
Dead	no	1 (1.8%)	4 (13.8%)	n.s.
Status after 365 days				
Dead	2 (3.9%)	4 (7.1%)	5 (17.2%)	n.s.

CPR: cardiopulmonary resuscitation; MAC score: 1 point for each of the following complications – low cardiac output syndrom, stroke, new need for renal replacement therapy, reintubation, IMC: intermediate care unit; HDU: high dependency unit. Data are given as median and (25/75) percentiles for continuous variables and as percentage for categorial variables. #: p<0.05 CPB-short versus AKI. *: p<0.05 CPB-short versus CPB-long. §: p<0.05 CPB-long versus AKI.

Table: 3 Plasma levels of biomarkers for acute kidney injury

Plasma	**T1 Baseline before surgey**			**T2 End of surgery**			**T3 First post-op day**			**T4 Second post-op day**			**T5 Third post-op day**		
Number of cases	No AKI n=107		AKI n=29	No AKI		AKI	No AKI		AKI	No AKI		AKI	No AKI		AKI
CPB-duration	short	long		short	long		short	long		short	long		short	long	
Creatinine [mg/dl]	0.88 (0.76-1)	0.9 (0.8-1)	1.0 #§ (0.83-1.2)	0.79 (0.67-0.9)	0.8 a (0.7-0.8)	0.91 (0.74-1)	0.98 (0.74-1.1)	0.97 a (0.83-1.1)	1.17 #§ (1-1.3)	0.81 (0.71-1)	0.96 * (0.8-1.1)	1.52 #§a (1-2)	0.82 (0.7-1.1)	0.88 (0.73-1)	1.5 #§a (1-2)
NGAL [ng/ml]	99.4 (78-124)	105 (89-126)	123 # (97-155)	370 a (287-498)	539 *a (380-641)	573 #a (416-665)	194 a (129-278)	204 a (161-295)	252 a (193-427)	127 a (105-170)	145 *a (119-188)	209 #§ (148-420)	111 (93-154)	121a (107-157)	249 #§a (138-334)

Plasma levels of markers for acute kidney injury in patients that did either develop postoperative renal dysfunction according to the Acute-Kidney-Injury Network criteria or not in relation to duration of cardiopulmonary bypass (CPB). Data are given as median and (25/75) percentiles. CPB-short: CPB-time < 118 (short); CPB-long: CPB-time ≥ 118 min (long). NGAL: Neutrophil-Gelatinase-Associated-Lipocalin. Mann-Whitney-U test and Wilcoxons's matched pairs test; as appropriate. [a] denotes a significant difference ($p < 0.05$) in comparison with baseline (T1); [#] denotes a significant difference ($p < 0.05$) for the CPB-short versus the AKI-group; [*]denotes a significant difference ($p < 0.05$) for CPB-short versus CPB-long; [§] denotes a significant difference ($p < 0.05$) for CPB-long versus AKI.

Table: 4 Urinary levels (adjusted for creatinine) of biomarkers for acute kidney injury

Urine	**T1 Baseline before surgey**			**T2 End of surgery**			**T3 6h post-OP**			**T4 12h post-OP**			**T5 24h post-OP**		
Number of cases	No AKI n=107		AKI n=29	No AKI		AKI	No AKI		AKI	No AKI		AKI	No AKI		AKI
CPB-duration	short	long		short	long		short	long		short	long		short	long	
NGAL [µg/g creatinine]	7.7 (5.8-12)	8.7 (4.2-15)	6.4 (3-13)	50 a (20.5-113)	439 *a§ (37-1180)	140 a (27-412)	21 a (12-48)	38.1 *a (17-82)	36.7 #a (22.4-126)	18.2 a (12-43)	31 a * (18.7-58)	33.3 a (19.5-53)	20.7 a (12-42.7)	24.4 a (12-38)	54 (37-70)
KIM-1 [ng/g creatinine]	0.72 (0.42-13)	0.89 (0.52-1.4)	0.83 (0.6-1.3)	0.65 (0.34-1.3)	1.4 *a§ (0.75-1.8)	0.76 (0.5-1.5)	1.03 (0.65-1.3)	1.04 (0.82-2)	1.3 #a (0.9-2)	1.7 a (1-2.3)	2.2 a (1.4-3.2)	1.8 a (1-2.9)	2.5 a (1.6-3.8)	3.6 a (1.9-5.7)	3.2 a (1.7-6.7)
L-FABP [µg/g creatinine]	3.95 (2-7)	4.56 (2.8-7)	7.3 # (3.8-9.6)	32.2 a (9-131)	191 *a§ (39-671)	54 a (22-664)	18.9 a (7.6-25)	26.9 *a (13.5-86)	19.3 a (7.8-78)	11.5 a (8.5-23)	25 *a (13.7-53)	25 #a (15.3-69)	9.9 a (5.6-18)	15.3 a (8.3-25)	22.7 *#a (9.2-76)

Urinary levels (adjusted for creatinine) of markers for acute kidney injury in patients that did either develop postoperative renal dysfunction according to the Acute-Kidney-Injury Network criteria or not in relation to duration of cardiopulmonary bypass (CPB). Data are given as median and (25/75) percentiles. CPB-short: CPB-time < 118 (short); CPB-long: CPB-time ≥ 118 min (long). NGAL: Neutrophil-Gelatinase-Associated-Lipocalin; KIM-1: Kidney-injury molecule -1; L-FABP: Liver fatty-acid binding protein. Mann-Whitney-U test and Wilcoxons's matched pairs test; as appropriate. [a] denotes a significant difference ($p < 0.05$) in comparison with baseline (T1); [#] denotes a significant difference ($p < 0.05$) for the CPB-short versus the AKI-group; [*] denotes a significant difference ($p < 0.05$) for CPB-short versus CPB-long; [§] denotes a significant difference ($p < 0.05$) for CPB-long versus AKI.

Table 5: Receiver operating characteristic analysis

Factor	Area-under-the curve (AUC)	95% CI	Significance *p-value*
Plasma			
NGAL (t1)	0.641	0.522-0.760	0.03
NGAL (t2)	0.619	0.499-0.739	0.08
NGAL (t3)	0.622	0.502-0.741	0.09
Creatinine (t1)	0.659	0.541-0.777	0.01
Creatinine (t2)	0.618	0.498-0.738	0.06
Creatinine (t3)	0.730	0.618-0.842	0.0004
Delta. Creatinine.t2-t1	0.598	0.477-0.718	0.12
Delta. Creatinine.t3-t1	0.688	0.572-0.804	0.004
Urine			
NGAL (t1)	0.565	0.444-0.685	0.37
NGAL (t2)	0.517	0.397-0.636	0.82
NGAL (t3)	0.605	0.484-0.725	0.13
L-FABP (t1)	0.661	0.543-0.779	0.02
L-FABP (t2)	0.524	0.404-0.644	0.72
L-FABP (t3)	0.518	0.398-0.637	0.79
KIM-I (t1)	0.526	0.407-0.646	0.69
KIM-I (t2)	0.533	0.413-0.653	0.61
KIM-I (t3)	0.600	0.479-0.720	0.14

AUC: Area under the Curve; NGAL: Neutrophil-Gelatinase-Associated-Lipocalin; KIM-1: Kidney-injury molecule-1; L-FABP: Liver fatty-acid binding protein; t1: before induction of anesthesia; t2: at the end of surgical procedure; t3: 6h after end of surgery.

Table 6: Univariate binary logistic regression for relevant factors possibly influencing CSA-AKI

Univariate analysis		
Factor	***OR (95% CI)***	***Significance p-value***
Demographic data		
Age (years)	1.06 (1.02 to 1.12)	0.02
Height (cm)	0.959 (0.913 to 1.005)	0.09
Weight (kg)	0.995 (0.996 to 1.02)	0.739
male	baseline	
female	1.63 (0.65 to 3.92)	0.273
Surgical-related data		
Duration surgery (min)	1.0029 (0.998 to 1.007)	0.14
Duration CPB (min)	1.0035 (0.9965 to 1.01)	0.29
Aortic cross-clamp time (min)	1.001989 (0.9928 to 1.01)	0.654
Surgical priority		
- elective	baseline	
- urgent	not applicable	
- emergency	1.66 (0.48 to 5.07)	0.384
Pre-operative data		
LVEF < 30%	baseline	
LVEF 30-50%	1.18 (0.14 to 24.94)	0.88
LVEF > 50%	1.05 (0.14 to 21.2)	0.964
eGFR § (ml/min/1.73m2)	0.982 (0.96 to 1.0005)	0.069
Risk stratification		
Logistic EuroSCORE	33.2 (1.28 to 846.7)	0.03
Laboratory analyses		
Delta creatine / [mg/dl]	0.96 (0.92 to 0.99)	0.03
L-FABP t:1 / [µg/g creatinine]	1.03 (0.99 to 1.08)	0.08
L-FABP t:2 / [µg/g creatinine]	0.999 (0.999-1.0001)	0.85
Plasma NGAL t:1 / [ng/ml]	1.01 (1.0 to 1.02)	0.03
Plasma NGAL t:2 / [ng/ml]	1.007 (1.001-1.02)	0.2
Multivariate analysis		
Age (years)	1.06 (1.02 to 1.12)	0.02

Logistic EuroSCORE: European System for Cardiac Risk Evaluation; NGAL: Neutrophil-Gelatinase-Associated-Lipocalin; L-FABP: Liver Fatty Acid Binding Protein; eGFR: estimated glomerular filtration rate; LVEF: left ventricular ejection fraction; CPB: cardiopulmonary bypas.

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Conflict of interest

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