Kidney & Blood Pressure Research

Kidney Blood Press Res 2017;42:456-467
DOI: 10.1159/000479298
Published online: July 28, 2017
Accepted: May 02, 2017

Original Paper

TIMP-2*IGFBP7 (Nephrocheck®) Measurements at Intensive Care Unit Admission After Cardiac Surgery are Predictive for Acute Kidney Injury Within 48 Hours

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Key Words
AKI • Nephrocheck • Cardiac Surgery • Epidemiology

Abstract
Background/Aims: Acute kidney injury (AKI) is a postoperative complication after cardiac surgery with a high impact on mortality and morbidity. Nephrocheck® [TIMP-2*IGFBP7] determines markers of tubular stress, which occurs prior to tubular damage. It is unknown at which time-point [TIMP-2*IGFBP7] measurement should be performed to ideally predict AKI. We investigated the association of [TIMP-2*IGFBP7] at various time-points with the incidence of AKI in patients undergoing elective cardiac surgery including cardio-pulmonary bypass. ***Methods:*** In a prospective cohort study, serial blood and urine samples were collected from 150 patients: pre-operative, at ICU-admission,24h and 48h post-surgery. AKI was defined as Serum-Creatinine rise >0.3 mg/dl within 48hrs. Urinary [TIMP-2*IGFBP7] was measured at pre-operative, ICU-admission and 24h post-surgery; medical staff was kept blinded to these results. ***Results:*** A total of 35 patients (23.5%) experienced AKI, with a higher incidence in those with high [TIMP-2*IGFBP7] values at ICU admission (57.1% vs. 10.1%, p<0.001). In logistic regression [TIMP-2*IGFBP7] at ICU admission was independently associated with the occurrence of AKI (Odds Ratio 11.83; p<0.001, C-statistic= 0.74) after adjustment for

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EuroSCORE II and CBP-time. ***Conclusions:*** Early detection of elevated [TIMP-2*IGFBP7] at ICU admission was strongly predictive for postoperative AKI and appeared to be more precise as compared to subsequent measurements.

Introduction

Acute kidney injury (AKI) is a common postoperative complication after cardiac surgery with a high impact on short- and long term mortality and morbidity [1-5]. According to current guideline recommendations, it is defined by a rise in serum creatinine (SCr) and/or decline in urine volume [6], however, these subsequent effects emerge with delay after the damage itself occurred [7]. Early detection of tubular stress/damage with regard to prediction of AKI may potentially help to prevent overt AKI by adjusting medical therapy. Other biomarkers of AKI (e.g. Cystatin C, NGAL) are of limited relevance for AKI prediction as they do not provide any gain of time over SCr [4-6, 8-11].

The Nephrocheck® system combines two tubular stress biomarkers – insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2) – for an early detection of tubular damage. Recent publications indicate that this method can detect AKI endangered patients in a very early state [7, 12-15]. Yet, it is unknown at which time-point [TIMP-2*IGFBP7] (Nephrocheck®) measurement should be performed to predict AKI in patients undergoing cardiac surgery [16-18].

The present study aims to investigate the predictive value of [TIMP-2*IGFBP7] measurements at various time points with the incidence of AKI within 48 hours after elective cardiac surgery. Furthermore, we investigated whether the predictive ability of [TIMP-2*IGFBP7] was increased if it was combined with routinely available preoperative and intraoperative factors.

Materials and Methods

The current study was part of a prospective cohort study to analyze the association of the Catechol-O-Methytransferase genotype with the incidence of AKI after cardiac surgery [19]. Adult patients were eligible if they were undergoing elective cardiac surgery (coronary artery bypass graft [CABG] with or without mammary artery bypass, valve surgery [reconstruction, replacement] with or without removal of the atrial auricle, combined CABG and valve surgery, or surgery of the thoracic aorta) involving cardiopulmonary bypass (CPB) at the Dept. of Cardiovascular Surgery, University Hospital Würzburg. Patients with advanced stages of chronic kidney disease (CKD, eGFR<60 ml/min/1.73m^2) were excluded as well as individuals with signs of active infection (clinical assessment), on medication with COMT inhibitors, MAO inhibitors or with immunosuppressive therapy and women during pregnancy and lactation. The study protocol and data handling was approved by the Ethics Committee of the Medical Faculty at the University of Würzburg [302/13] and the data protection officer at the University Hospital Würzburg.

Patients were enrolled prior to surgery (preferably at the day of admission) and details of patient characteristics, medical history, medication and physical examinations were collected. Furthermore, data of clinical routine preoperatively, during anesthesia and surgery and during the postoperative period until hospital discharge were collected, in parts supported by the CHFC DataWarehouse [20].

Study specific collection of biomaterials included: serum and EDTA blood and spot urine at baseline (introduction of anesthesia, which is about 0.5 hours prior to the beginning of surgery and about 1.0 hour prior to the start of CPB), and serum and urine at four time-points post-surgery (ICU-admission directly after surgery, 24hrs post-surgery/in the early morning hours of the first post-operative day, in the morning hours of the second post-operative day/48 hrs post-surgery and 6 days post-surgery/discharge). Tubes for blood and urine sampling were prepared by study personnel, were collected/drawn by nurses/physicians during clinical routine and were processed thereafter by study personnel according to standardized protocols.

[TIMP-2*IGFBP7] was measured at baseline, ICU-admission, and 24hrs post-surgery in urinary samples according to manufacturer's instructions. Urine samples were analyzed within 2 hours using Nephrocheck® (Astute Medical, San Diego, CA, USA), based on florescence labeled immunoassay. Quality controls were provided by the manufacturer. After measuring [TIMP-2] and [IGFBP7] concentrations, the signals are calculated into a single metric number. Values of ≥ 0.3 indicate an elevated risk for AKI. Of note, the treating physicians/nurses were kept blinded to the [TIMP-2*IGFBP7] results.

The primary endpoint was defined as the development of AKI within the first 48 hours after surgery according to current guideline recommendations [6]; at least increase in SCr ≥0.3 mg/dl within 48hrs or increase in SCr ≥1.5 times baseline or reduction of urine volume <0.5 ml/kg/h for at least 6 hr. Secondary outcomes comprised stages of AKI within 48 hours; SCr increase of ≥0.3 mg/dl or SCr-increase of 1.5-1.99 times baseline (stage 1), SCr-increase of 2.0-2.9x baseline SCr (stage 2) and SCr-increase ≥3.0x baseline or SCr >4 mg/dl or dialysis (stage 3). Of note, time-distance from baseline to respective SCr measurements was verified by time-stamps of laboratory analyses. Operative risk based on the patient's preoperative status was assessed by EuroSCORE II including information on age, gender, chronic pulmonary disease, extracardiac arteriopathy, neurological dysfunction disease, previous cardiac surgery, kidney function, active endocarditis (which represented an exclusion criterion for the current study) and critical preoperative state [21].

Statistical methods

Patient demographics are presented as mean and standard deviation (SD), median and interquartile range (IQR) and number of observations with proportions (%), as appropriate. Differences across groups of AKI risk by [TIMP-2*IGFBP7] were assessed by t-test, Mann-Whitney-U-Test and χ^2-test/Fisher's exact test, respectively. The association of [TIMP-2*IGFBP7] categories (i.e. ≤0.3 / >0.3) at baseline, ICU admission, and 24hrs post-surgery with the risk of AKI within 48 hrs post-surgery was determined by logistic regression. Based on these univariate results, baseline kidney function, EuroSCORE II, CPB-time and baseline-[TIMP-2*IGFBP7] values were tested in stepwise multivariate logistic regression along with [TIMP-2*IGFBP7] values at ICU-admission (the most important predictor in univariate analysis) regarding independent relationships to AKI. The prognostic value of each variable and the multivariate model was described by C-statistics. Additionally, we provided operating characteristic curves (ROC) aligned with 95% confidence intervals (CI) drawn out of the multivariate analyses. In sensitivity analyses, we replaced missing [TIMP-2*IGFBP7] -values at ICU-admission by [TIMP-2*IGFBP7] -values of 24h post-surgery (n=35 of 50), if no AKI was diagnosed until this time-point. We also tested [TIMP-2*IGFBP7] values as continuous variable rather than categorized at 0.3. Two-sided p-values of ≤0.05 were considered as statistically significant. Statistical analyses were performed using SPSS Version 23 and SAS version 9.3.

Results

A total of 150 subjects (median 67 yrs old, 28% female) were enrolled in the study between April and December 2014 [19]. 35 patients (23.5%) experienced AKI within 48h after surgery. Hereof 27 (77.1%) were of stage 1, 6 (17.1%) of stage 2 and 2 (5.7%) were stage 3 [19]. [TIMP-2*IGFBP7] measurements were available in 95.3% at baseline (median 0.39, IQR 0.14 to 1.20), 66.7% at ICU-admission after surgery (median 0.08, IQR 0.04 to 0.27), and in 98.7% patients 24hrs post-surgery (median 0.21, IQR 0.11 to 0.36), respectively (table 1).

[TIMP-2*IGFBP7] values >0.3 at ICU admission were strongly associated with the incidence of AKI (OR 11.8, p<0.001) with a sensitivity of 0.60 and a specificity of 0.88. Measurement 24hrs post-surgery approached significance (OR 2.11, p=0.06, sensitivity 0.60, specificity 0.69) and measurements directly before surgery (baseline) were not related with the risk of AKI (OR 0.76, p=0.53) (table 1). Positive predictive value (PPV) was 57.1%, while the negative predictive value (NPV) was 89.9%.

Based on these results, patient characteristics are displayed according to [TIMP-2*IGFBP7] categories at ICU admission (table 2; see table 3 for patient characteristics according to [TIMP-2*IGFBP7] categories at baseline). Patients with [TIMP-2*IGFBP7] values >0.3 at post-operative ICU-admission were less likely after CABG surgery

Table 1. [TIMP-2*IGFBP7] categories and incidence of AKI within 48hrs after surgery

[TIMP-2*IGFBP7]	N (%) with available measurement	No AKI		AKI		OR* (95% CI)	p-value	C-statistic
		≤0.3	>0.3	≤0.3	>0.3			
Baseline	143 (95.3)	46 (41.8)	64 (58.2)	16 (48.5)	17 (51.5)	0.76 (0.35; 1.67)	0.50	0.533
ICU-admission	100 (66.7)	71 (88.8)	9 (11.2)	8 (40.0)	12 (60.0)	11.83 (3.82; 36.7)	<0.001	0.744
24hrs post-surgery	148 (98.7)	78 (69.0)	35 (31.0)	18 (51.4)	17 (48.6)	2.11 (0.97; 4.56)	0.059	0.588

data are numbers of observations (%) and Odds ratio* (OR) with 95% confidence interval (CI) resulting from univariate logistic regression modelling AKI within 48h; abbreviations: AKI, acute kidney injury; ICU, intensive care unit.

Table 2. Patient characteristics according to [TIMP-2*IGFBP7] categories at ICU-admission

Variable	[TIMP-2*IGFBP7] ≤0.3 (n=79)	[TIMP-2*IGFBP7] >0.3 (n=21)	P-value
Age [yr]	66 (60; 75)	71 (63; 77)	0.13
Female	22 (27.8)	8 (36.4)	0.44
Medical history			
History of heart failure	40 (54.1)	6 (30.0)	0.06
History of stroke/TIA	19 (25.3)	6 (31.6)	0.58
Atrial fibrillation	8 (10.7)	3 (14.3)	0.70
Pacemaker	1 (1.3)	4 (19.0)	0.008
EuroSCORE II [%]	1.68 (0.92; 2.78)	1.75 (1.00; 3.06)	0.71
Measurements/laboratory			
Weight [kg]	82.2±15.2	81.4±17.8	0.84
Height [cm]	173±9	172±8	0.66
FEV ≤79%	19 (30.6)	4 (28.6)	1.00
Creatinine [mg/dl]	0.89 (0.73; 1.08)	1.02 (0.75; 1.18)	0.26
eGFR [ml/min/1.73m^2]	84.6 (69.6; 96.1)	75.5 (58.0; 89.7)	0.23
Surgery			
Coronary Artery Bypass Graft	43 (54.4)	5 (22.7)	0.008
Valve	44 (55.7)	15 (68.2)	0.29
Aortic surgery	42 (53.8)	15 (68.2)	0.23
OP duration [h:min]	3:29 (3:00; 4:03)	3:40 (3:17; 4:22)	0.13
Time on CPB [h:min]	1:34 (1:18; 2:02)	2:00 (1:47; 2:23)	0.003
X-Clamp	78 (98.7)	20 (90.9)	0.12
Circulatory arrest	8 (11.9)	0 (0)	0.19
intra/early postoperative outcomes			
Operative Complication[a]	3 (3.8)	3 (13.6)	0.12
Transfusion	11 (14.1)	2 (9.1)	0.73
AKI stages[b] within 48 hrs			<0.001
- Stage 1	8 (10.1)	8 (38.1)	
- Stage 2	0	4 (19.1)	
- Stage 3	0	0	

data are mean ± standard deviation (SD), or median (interquartile range IQR) or numbers of observations (%). Abbreviations: TIA, transient ischemic attac; FEV, forced expiratory volume; SCr, serum creatinine; eGFR, estimated glomerular filtration rate; AKI, acute kidney injury. [a] cumulation of: second x-clamping; Left ventricular assist device; extensive bleeding, [b] stages of AKI within 48 hrs; SCr increase ≥0.3 mg/dl or 1.5-1.99x baseline (stage 1), SCr-increase 2.0-2.9x baseline SCr (stage 2); SCr-increase ≥3.0x baseline or SCr >4 mg/dl or dialysis (stage 3).

(54.4% vs. 22.7%; p=0.008) and had a longer time on CPB (1:59 h vs. 1:34h, p=0.01), as compared to subjects with [TIMP-2*IGFBP7] values ≤0.3. The latter patients were less likely to require preoperative pacemaker treatment (19.0% vs. 1.3%, p=0.008), but tended to be more likely to have a history of heart failure (54.1% vs. 30.0%; p=0.06). Eight patients with [TIMP-2*IGFBP7] values ≤0.3 experienced AKI within 48 hrs, which were exclusively of AKI stage 1.

In logistic regression modelling, we analyzed the relationship of pre-operative and intraoperative variables with AKI within 48h post-surgery as pre-specified by the analysis plan. These were, however, not related to the outcome in univariate analyses: baseline eGFR (OR 0.99; IQR 0.97 to 1.01; C-statistic 0.58),

Table 3. Patient characteristics according to [TIMP-2*IGFBP7] categories at baseline

Variable	[TIMP-2*IGFBP7] ≤0.3 (n=62)	[TIMP-2*IGFBP7] >0.3 (n=81)	P-value
Age [vr] – median (IQR)	71 (64 to 76)	65 (59 to 73)	0.01
Female – n (%)	22 (35.5)	15 (18.5)	0.02
Weight [kg] – mean±SD	80.8±16.9	83.8±14.5	0.26
Height [cm] – mean±SD	172±9	173±8	0.70
Heart failure – n (%)	31 (52.5)	37 (49.3)	0.71
Atrial fibrillation – n (%)	7 (11.9)	9 (11.5)	0.95
Pacemaker – n (%)	2 (3.4)	5 (6.4)	0.70
History of cerebrovascular disease – n (%)	19 (31.7)	18 (24.0)	0.32
Creatinine [mg/dl] – median (IQR)	0.94 (0.80 to 1.22)	0.89 (0.75 to 1.02)	0.03
EuroSCORE II [%] – median (IQR)	2.04 (1.16 to 3.55)	1.28 (0.86 to 2.52)	0.005
FEV ≤ 79% – n (%)	13 (26.0)	20 (35.1)	0.31
Nephrocheck – median (IQR)			
Baseline	0.13 (0.10 to 0.20)	1.15 (0.49 to 1.69)	
ICU	0.07 (0.04 to 0.22)	0.08 (0.04 to 0.28)	
24h	0.17 (0.10 to 0.34)	0.23 (0.11 to 0.36)	
48h	0.28 (0.19 to 0.80)	0.57 (0.21 to 0.97)	
Acute kidney injury within 48h – n (%)	16 (25.8)	17 (21.0)	0.50
Stage 1	11 (68.8)	15 (88.2)	0.91
Stage 2	3 (18.8)	2 (11.8)	0.65
Stage 3	2 (12.5)	0 (0)	0.19

Table 4. Determinants of AKI within 48 h after cardiac surgery (multivariate logistic regression)

	Model 1		Model 2		Model 3*	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
$[TIMP\text{-}2*IGFBP7]_{ICU}$ (>0.3)	11.7 (3.74; 36.4)	<0.001	14.01 (4.22; 46.5)	<0.001	11.7 (3.60; 38.13)	<0.001
EuroSCORE II [log(%)]	0.93 (0.43; 2.01)	0.85	--		--	
$eGFR_{baseline}$ (ml/min/1.73m^2)	0.99 (0.97; 1.02)	0.74	--		--	
CPB-time [log (h)]	--		0.76 (0.16; 3.66)	0.73	--	
$[TIMP\text{-}2*IGFBP7]_{baseline}$ (>0.3)	--		--		1.09 (0.32; 3.65)	0.89
C-statistic	0.772		0.744		0.745	

data are Odds Ratios (OR) with 95% confidence interval (CI) of stepwise multivariate logistic regression; abbreviations: AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; CPB, cardio-pulmonary bypass. *Similar results if the interaction of $[TIMP\text{-}2*IGFBP7]_{ICU}$ category and $[TIMP\text{-}2*IGFBP7]_{baseline}$ category was included in the model, which itself was not significant (p=0.81).

EUROSCORE 2 (log) (OR 1.49; IQR 0.95 to 2.37, C-statistic 0.59) and time on CPB (log) (OR 2.38; IQR 0.85 to 6.64, C-statistic 0.59). Also in multivariate modelling (table 4), no further significant associations could be observed (table 4), neither by considering pre-operative patient characteristics (model 1), time on CPB (model 2) or baseline [TIMP-2*IGFBP7] category (model 3) and its interaction with [TIMP-2*IGFBP7] at ICU admission. Thus, the

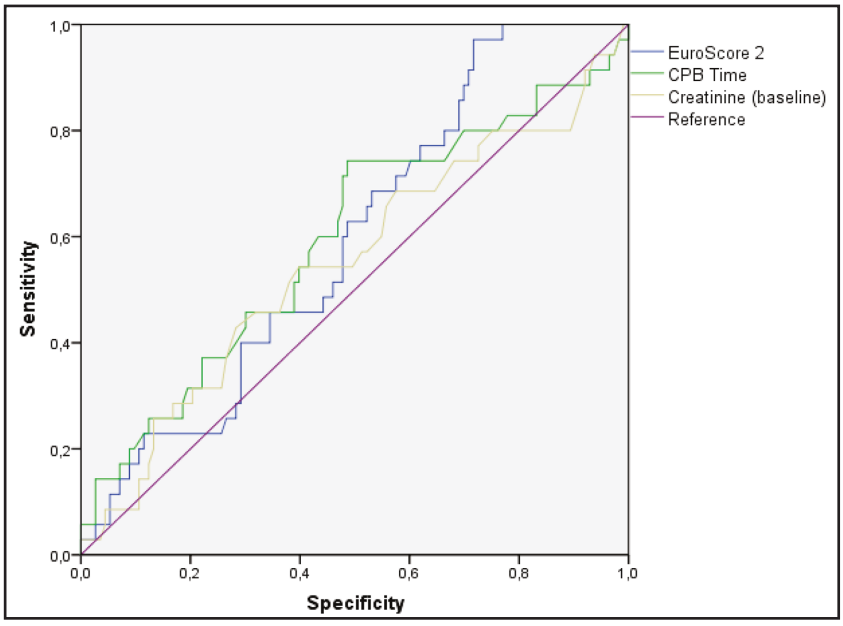

Fig. 1. ROC analyses for EuroScore 2, CPB Time and baseline creatine. CPB: cardiopulmonary bypass.

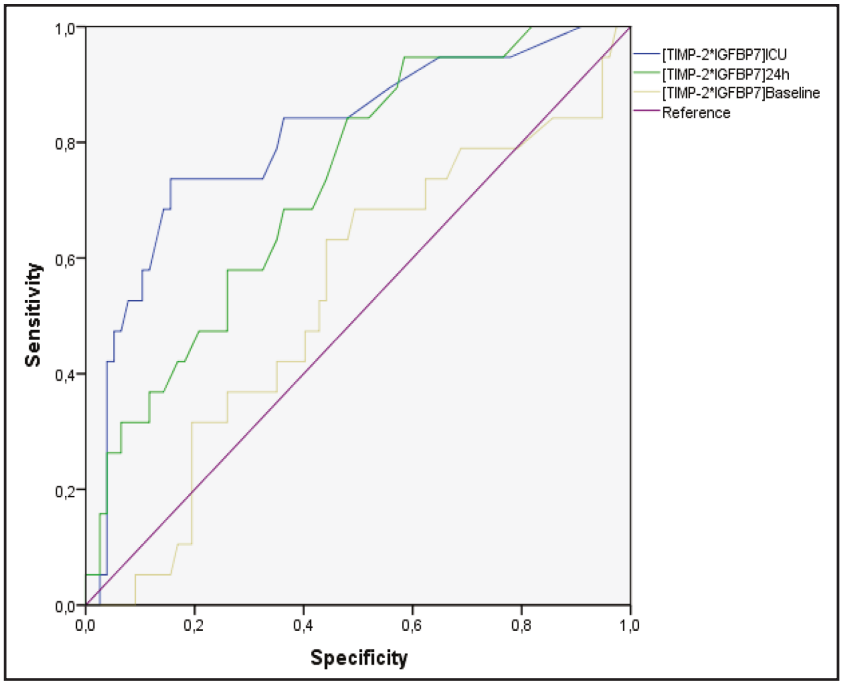

Fig. 2. ROC analyses for [TIMP-2*IGFBP7] measurements.

[TIMP-2*IGFBP7] category at ICU admission was the only significant determinant of AKI within 48hrs (table 1, table 4) with considerable predictive accuracy (C-statistic >0.74). For better visualization of our multivariate models we additionally provided ROC curves (figure 1, figure 2).

In addition, we investigated log-transformed [TIMP-2*IGFBP7] values at ICU admission rather than its categories (table 5). In univariate analysis, [TIMP-2*IGFBP7] values at ICU admission were strongly associated with the risk of AKI (OR 2.43, 95% CI 1.57 to 3.77) with even higher predictive accuracy (C-statistic 0.81). Similar results emerged from the multivariate models, with no significant associations of the further tested variables.

In a final sensitivity analysis, we replaced (n=35 of 50) missing [TIMP-2*IGFBP7] values

Table 5. Determinants of AKI within 48 h after cardiac surgery (multivariate logistic regression, [TIMP-2*IGFBP7] values as continuous variable)

	Model 1		Model 2		Model 3*	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
$[TIMP\text{-}2*IGFBP7]_{ICU}$ [log(Unit)]	2.44 (1.56; 3.79)	<0.001	2.86 (1.73; 4.73)	<0.001	2.6 (1.64; 4.29)	<0.001
EUROSCORE 2 [log(%)]	0.88 (0.40; 1.91)	0.74	--		--	
$eGFR_{baseline}$ (ml/min/1.73m²)	0.99 (0.96; 1.02)	0.59	--		--	
CPB-time [log (h)]	--		0.48 (0.09; 2.50)	0.39	--	
$[TIMP\text{-}2*IGFBP7]_{baseline}$ [log(Unit)]	--		--		0.90 (0.58; 1.42)	0.66
C-statistic	0.820		0.821		0.818	

data are Odds Ratios (OR) with 95% confidence interval (CI) of stepwise multivariate logistic regression; abbreviations: AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; CPB, cardio-pulmonary bypass. *Similar results if the interaction of $[TIMP\text{-}2*IGFBP7]_{ICU}$ and $[TIMP\text{-}2*IGFBP7]_{baseline}$ was included in the model, which itself was not significant (p=0.87).

Table 6. Determinants of AKI within 48 h after cardiac surgery (multivariate logistic regression, [TIMP-2*IGFBP7] values as categorical variable; missing $[TIMP\text{-}2*IGFBP7]_{ICU}$ values replaced by $[TIMP\text{-}2*IGFBP7]_{24\ hrs}$)

	Model 1		Model 2		Model 3*	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
$[TIMP\text{-}2*IGFBP7]_{ICU}$ [>0.3]	5.69 (2.16; 15.0)	<0.001	5.86 (2.22; 15.5)	<0.001	5.54 (2.06; 14.9)	<0.001
EUROSCORE 2 [log(%)]	0.88 (0.50; 2.02)	0.99	--		--	
$eGFR_{baseline}$ (ml/min/1.73m²)	0.99 (0.97; 1.02)	0.75	--		--	
CPB-time [log (h)]	--		1.15 (0.30; 4.44)	0.84	--	
$[TIMP\text{-}2*IGFBP7]_{baseline}$ [>0.3]	--		--		1.34 (0.48; 3.77)	0.58
C-statistic	0.690		0.707		0.707	

data are Odds Ratios (OR) with 95% confidence interval (CI) of stepwise multivariate logistic regression; abbreviations: AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; CPB, cardio-pulmonary bypass. *Similar results if the interaction of $Nephrocheck_{ICU}$ category and $Nephrocheck®_{baseline}$ category was included in the model, which itself was not significant (p=0.87).

at ICU admission by [TIMP-2*IGFBP7] values if no AKI had occurred at this time-point. Overall similar associations were found (table 6), however with less strong association and predictive accuracy.

Discussion

Our results demonstrate that in patients undergoing cardiac surgery, measurement of [TIMP-2*IGFBP7] at ICU admission directly after surgery is a strong and accurate predictor of AKI within 48 hours after surgery. No further clinical factors such as pre-operative kidney function, operative risk by EuroSCORE II or time on CPB could add more information to our models. Measurement of [TIMP-2*IGFBP7] before surgery was not helpful to predict AKI,

neither alone nor in combination with [TIMP-2*IGFBP7] values at ICU admission. AKI is a common complication after cardiac surgery with an incidence between 20-50% in patients undergoing cardiac surgery [2, 3, 22-25]. Stage I of AKI according to most recent guidelines is defined as a rise in SCr of ≥0.3mg/dl within 48 hours. This definition appears to be very strict, but recent literature indicates that AKI even in stage I carries a high impact on short- and long-term prognosis of patients. Patients experiencing AKI have a higher risk of mortality, readmission rates and a higher risk for developing heart failure as compared to patients without AKI [4, 5, 22, 23, 26, 27]. Using SCr or drop in urine volume to diagnose AKI is problematic, because both markers develop after tubular damage has occurred [7]. Early detection or even prediction of AKI is crucial for nephroprotective therapy. Some established biomarkers (NGAL, Cystatine C) elevate earlier and more specific than SCr, but still any time advantage over SCr is insufficient to prevent AKI [7]. Early onset of therapy is associated with lower mortality after AKI [6, 11, 28].

Nephrocheck® is a bedside test that calculates a numeric value based on the measurements of two urinary parameters [TIMP-2*IGFBP7]. Based on immunofluorescence labeling, renal injury can be detected in an early state [7, 11, 13-15, 29]. The dynamics seem to be dependent on the cause, severity as well as the recovery of AKI [7, 17]. After CABG surgery, measurement of [TIMP-2*IGFBP7] predicted AKI in smaller cohorts [16-18]. Wetz et al. could not demonstrate any association of early [TIMP-2*IGFBP7] measurements and postoperative AKI [18]. That cohort solely consisted of CABG patients, while the present cohort included patients with aortic- and mitral valve disease. There are several key differences: patients with CABG had a lower risk to develop AKI in our cohort and those patients undergoing valve surgery had a longer time on CPB than CABG patients. As CPB-time was a significant determinant of intraoperative kidney injury, the causes for AKI in our study might be intraoperative, which would explain the difference in the predictive value of the early postoperative measurement [18].

Some patients were tested with a high risk for AKI, i.e. [TIMP-2*IGFBP7] >0.3, at ICU admission, but did not experience AKI within 48 hours. These false positive results may occur due to the specificity of the test itself, but may also represent treatment bias. Clinical therapy and management was not influenced neither by our study protocol nor by [TIMP-2*IGFBP7] measurement, as the treating physicians were kept blinded to these results. However, other factors, laboratory constellations or measurements may had been interpreted as early clinical signs of AKI which in consequence might have caused treatment decisions on the ICU that counteracted the occurrence of AKI (e.g. volume therapy, dose adjustments of medication etc.) which resulted in lower specificity of the test.

Since its introduction in 2011, EuroSCORE II has widely been used to assess the patients' operative mortality risk in cardiac surgery, based on routinely available preoperative markers and patient characteristics [21]. It considers age, gender, renal impairment, extracardiac arteriopathy, mobility, previous cardiac surgery, chronic lung disease, acute endocarditis, diabetes, the preoperative clinical state, NYHA and CCS classification, LV-function and the weight of the procedure for the calculation. Therefore, it is an established tool to describe the patient's preoperative condition and the weight of the procedure. For the course of the operation, the time on CPB time is an important surrogate marker for subsequent outcome [30]. It is directly dependent on the complexity of the procedure undertaken, but also on intraoperative complications, extraordinary situations or other adverse events. Furthermore, CPB time is associated not only with the incidence of AKI after but it is also associated with mortality after cardiac surgery [30-34]. Therefore, we adjusted our multivariate model with CBP time and EuroSCORE II to sum up pre- and intraoperative data into a model without using to many individual variables. Yet, [TIMP-2*IGFBP7] values at ICU admission remained strongly associated with the incidence of AKI within 48 hours after cardiac surgery. Therefore, [TIMP-2*IGFBP7] measurement adds information about the risk of AKI aside from preoperative and intraoperative factors, and does not only identify high risk patients with a suboptimal or complex operation.

We also tested [TIMP-2*IGFBP7] values as continuous variable rather than the suggested categories (i.e. cut-off >0.3) and found a strong association of [TIMP-2*IGFBP7] results with the risk of AKI. This relationship was even of higher accuracy than the categorical variable, indicated by a higher value of the C-statistic. However, according to our results, the suggested cut-off of >0.3 seems to be useful and applicable to clinical practice. Of note, we did not investigate other cut-off values for [TIMP-2*IGFBP7], which would have been beyond the scope of the current manuscript. In earlier studies, higher specificities were observed in high risk patients for AKI [17]. To raise the specificity of the [TIMP-2*IGFBP7] measurement, a pre-screening for high risk patients with risk scores before performing the test seems to be a reasonable way in clinical use of the test.

Although [TIMP-2*IGFBP7] measurement at ICU admission was strongly related to the outcome, we have to acknowledge that a substantial proportion of patients (33%) did not have valid values at this time-point. This was mainly because of two reasons: first, urine samples were collected by the ICU staff (nurses/physicians) during routine clinical care. If it was decided that no laboratory was measured at ICU admission (e.g. outstandingly stable conditions of the patient, or short time-distance to the regular morning laboratory collections) then also no urine sample for study purposes was collected. Second, hematuria interferes with [TIMP-2*IGFBP7] measurement. Hematuria is not an uncommon complication directly after surgery and is frequently being caused by anticoagulation therapy. Therefore, only a part of all patients developing an AKI had a [TIMP-2*IGFBP7] measurement on ICU admission. However, not only that measurement of [TIMP-2*IGFBP7] was impossible in these patients, the risk of AKI may have been affected by specific therapy for hematuria, which might include volume therapy, which has beneficial renal effects. We approached these effects by replacing missing values at ICU admission by values 24 hours after surgery in those patients who did not experience AKI by this time-point. [TIMP-2*IGFBP7] of >0.3 were still related to an increased risk for AKI, independently of clinical factors, but the strength of the relationship and the accuracy of the model were significantly lower. We interpret this finding by the fact, that [TIMP-2*IGFBP7] measurement might be particularly helpful very early after damage to the kidney and thus very early during the development of overt AKI.

Aside from the already described potential of treatment bias, which was independent from the study protocol itself and independent from [TIMP-2*IGFBP7] measurement as these results were hidden from clinical routine, we have to mention other limitations of the current study. The limited sample size did not allow for extensive adjustments in multivariate modelling, therefore our results need to be interpreted with caution and warrant confirmation in independent, preferably larger studies. However, our study represents the largest prospective cohort on this topic in cardiac surgery. Finally, as we excluded patients with advanced CKD (i.e. eGFR <30 ml/min/1.73m^2), which are *per se* at high risk for AKI, we cannot claim any usefulness of [TIMP-2*IGFBP7] measurement in these patients.

Conclusions

[TIMP-2*IGFBP7] measurements at ICU admission directly after cardiac surgery predicts AKI within 48hrs after surgery independent from and more specific then clinical factors such as preoperative kidney function, EuroSCORE II and time on CPB. Early measurements such as at ICU admission appeared to be more precise as compared to later measurements. Further research on this promising method on larger and non-selected cohorts are needed to describe the entire prognostic power of [TIMP-2*IGFBP7] in detail and to transfer it into clinical use to prevent AKI [35].

Disclosure Statement

The Authors have no conflict of interest to declare that is relevant to this manuscript.

PUH reports research grants from German Ministry of Research and Education, European Union, Charité–Universitätsmedizin Berlin, Berlin Chamber of Physicians, German Parkinson Society, German Heart Foundation, University Hospital Würzburg, Robert Koch Institute, Charité–Universitätsmedizin Berlin (within Mondafis; supported by an unrestricted research grant to the Charité from Bayer), grants from University Göttingen (within FIND-AF randomized; supported by an unrestricted research grant to the University Göttingen from Boehringer-Ingelheim), grants from University Hospital Heidelberg (within RASUNOA-prime; supported by an unrestricted research grant to the University Hospital Heidelberg from Bayer, BMS, Boehringer-Ingelheim, Daiichi Sankyo), outside the submitted work.

Acknowledgements

We thank all patients providing data to the current study as we as we thank the physicians and study personnel (M. Lazariotou; J. Ullrich) for data collection and data management (T. Ludwig). We also acknowledge the support of the CHFC DataWarehouse (G. Fette) for data transfer of parameters of clinical routine. Parts of the results will be presented at the 46th annual conference of the German Society for Thoracic and Cardiovascular Surgery in February 2017 in Leipzig, Germany.

This study was supported by the German Ministry of Education and Research (BMBF) within the setting of the Comprehensive Heart Failure Center Würzburg (BMBF 01EO1004).

Nephrocheck® measurement kits were purchased from Astute Medical. “Astute Medical” had no influence on the conduct of the study, the analysis plan not in the interpretation of the results. All data are owned by the authors.

"This publication was supported by the Open Access Publication Fund of the University of Würzburg."

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