Original Article

Rapid Detection of Acute Kidney Injury by Urinary Neutrophil Gelatinase-Associated Lipocalin in Patients Undergoing Cardiopulmonary Bypass

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Abstract

Objective: Acute kidney injury (AKI) is common following cardiopulmonary bypass (CPB). The aim of this study is to determine the accuracy of urinary neutrophil gelatinase-associated lipocalin (NGAL) levels following cardiac surgery to establish the severity of renal impairment as compared to serum creatinine levels.

Methods: A total number of 28 patients undergoing elective cardiopulmonary bypass were included. Diagnostic criteria of AKI was established in case of a percentage increase in serum creatinine concentration of >50%. Serum creatinine levels were recorded in the preoperative period before induction and in the postoperative period at 24, 48, and 72 hours. Urinary NGAL measurement was performed before induction and in the 4th postoperative hour. The duration of CPB surgery, hospital stay, and cross-clamp time were recorded.

Results: Based on AKI criteria, subjects were grouped as AKI (n=11) and no AKI (n=19). Postoperative urinary NGAL levels were significantly higher in the group with AKI (11.8 ng mL^{-1} vs. 104.0 ng mL^{-1}, p=0.003). In the AKI group, CPB time bypass (111.9 min vs. 82.7 min) and cross-clamp time (76.9 min vs. 59.1 min) were significantly higher. A cut-off of 25.5 ng mL^{-1} yielded a sensitivity of 81.82% and a specificity of 94.12% at the postoperative 4th hour with an AUC of 0.947 for predication of AKI.

Conclusion: Urine NGAL rose significantly much earlier as compared to serum creatinine levels in the early postoperative period. Although larger case series are needed, we are of the opinion that urinary NGAL measurements may be used as an early clinical marker of AKI following CPB.

Key Words: Acute kidney injury, neutrophil gelatinase-associated lipocalin, cardiopulmonary bypass

Introduction

Approximately 30% of the patients undergoing cardiopulmonary bypass (CPB) surgery develop severe acute kidney injury (AKI) (1), and 1% of these patients require dialysis. Delays in the diagnosis of AKI increase morbidity and mortality (2). Taking emergency measures and adequate treatment can only be possible with early diagnosis of AKI.

Measurement of serum creatinine levels currently used to identify AKI has been found to be unreliable to identify renal dysfunction early after surgery (3). While neutrophil gelatinase-associated lipocalin (NGAL), identified primarily in activated neutrophils, is at low concentrations in normal conditions, it increases considerably in serum and urine when AKI develops (4, 5).

Urinary NGAL levels increase 24-48 hours before serum creatinine levels (6). NGAL levels are increased approximately 15 fold 2 hours after CPB, and 25 fold after 4 hours. The measured urine NGAL concentrations 4 hours after cardiac surgery, shows 91% sensitivity and 91% specificity for AKI detection if a cut-off value of 100 ng mL^{-1} is used (7). Therefore, urine NGAL measurement is considered to be superior to serum creatinine measurement, especially for intensive care patients (8). However, there is no consensus on the cut-off value of NGAL for clinical use. Further prospective randomized trials on different patient groups are needed (9).

The aim of this present study is to compare serum creatinine and urine NGAL levels in determining the severity of renal injury in patients operated on with CPB and to determine appropriate cut-off values for determining AKI developing after CPB.

Methods

This prospective clinical study was performed after approval from hospital Ethics committee (Bilim University Hospitals Clinical researches Ethics committee, date: 23.05.2013, no: 06-52) and written informed consents from all patients were obtained before the surgery. The study included 28 patients between 25 and 75 years of age, undergoing elective CPB surgery. Exclusion criteria were acute or chronic renal insufficiency, chronic obstructive pulmonary disease, congestive heart failure, use of angiotensin converting enzyme inhibitors, diuretics and nephrotoxic drugs, and history of myocardial infarction in the last 6 months. Respiratory function tests, blood coagulation tests, biochemistry tests and complete blood count of the patients were performed in the preoperative period and glomerular filtration rate and body mass index were calculated. Durations of CPB, aortic cross clamp, and intubation, length of intensive care and hospital stay, perioperative and postoperative haemodynamic parameters, serum creatinine levels at postoperative 24, 48 and 72 hours were recorded. A 50% increase in creatinine levels compared to baseline is considered as AKI (10, 11).

After IV line was established with an 18G catheter, patients underwent electrocardiogram, peripheral oxygen saturation and invasive arterial blood pressure monitoring with a 20G catheter placed in the radial artery in the operating room. Induction of anaesthesia was performed using sodium thiopental 4-7 mg kg^{-1}, fentanyl 0.1 mcg kg^{-1} and vecuronium 0.15 mg kg^{-1} iv. Patients were not preoxygenated. Anaesthesia was maintained with 1 MAC isoflurane in 50% oxygen/50% air mixture, vecuronium, propofol and fentanyl infusion. Right jugular vein catheterization was performed with a 7F catheter and continuous central venous pressure monitoring was performed.

After orotracheal intubation, patients were ventilated with 8-10 mL kg^{-1} tidal volume, 5 cmH$_2$O PEEP and end tidal CO$_2$ pressure 32-35 mmHg. After arterial catheterization, blood gas analysis was performed just before induction and at 30 minutes intervals perioperatively. Blood gas parameters were recorded at each hour in the postoperative first day and at 4 hour intervals after 24 hours. Activated clotting time (ACT) was recorded at 5 different time points. T1: just before the induction, T2: at 2 minutes after systemic heparinization, T3: at 30 minutes of perfusion T4: at 1 hours of perfusion, T5: after protamine was given.

Preoperative serum creatinine and urinary NGAL levels were measured in the morning of the surgery, before the induction of anaesthesia. Urine samples that should be collected at 4 hours after CPB were collected in the intensive care unit. The 24, 48 and 72 hours blood samples were collected in the intensive care unit or ward. 10 mL of fresh urine was centrifuged at 2500 rpm for 10 minutes. Urine samples were kept at -80°C until analysis (12). ELISA commercial kit (BioPorto Diagnostics, Denmark) was used to measure urinary NGAL levels. The enzymatic reactions were quantified in an automatic microplate photometer (Biotek, USA). NGAL concentrations are given in ng mL^{-1}.

Statistical analysis

Statistical Package for the Social Sciences, (SPSS Inc., Chicago, IL, USA) software program version 17.0 and Med Calc program version 11.3.3.0 were used to analyse study data. Descriptive statistics are given as frequency, percentage, mean, and standard deviation. Pearson Chi-square test and Fisher Exact test was used to compare qualitative data. In the comparison of quantitative data, Mann-Whitney U test was used in inter-group comparisons in the presence of two groups. Intra-group comparisons of creatinine and NGAL levels were done using Wilcoxon signed-rank test. ROC curve was used in predicting a cut-off value for serum creatinine and urinary NGAL levels in acute kidney injury. The results were evaluated with a 95% confidence interval, p<0.05 significance level and p<0.01 p<0.001 significance level in advance.

Results

The present study included 28 patients (67.9% males, 32.1% females) with a mean age of 58.8±13.5 years, mean body mass index of 27.9±7.1 kg m^2, and mean body surface area of 1.9±0.2 m^2. AKI developed in 11 (39.3%) of 28 patients. Concurrent diseases were diabetes mellitus (DM) in 7 (25.0%), and hypertension in 20 (71.4%) patients. The surgery types of the patients were as follows, Coronary Artery Bypass Grafting (CABG) in 21 (75.0%), CABG and valve replacement in 3 (10.7%), valve replacement in 3 (10.7%), valve replacement and aortic repair surgery in 1 (3.6%). The mean CPB duration was 94.2±38.8 min, mean cross clamp duration was 66.1±29.7 min, and length of surgical intensive care stay was 35.8±23.7 hours. The mean intubation duration of the patients was 7.4±3.4 hours. The mean length of ward stay was 5.2±1.2 days.

Postoperative serum creatinine concentrations of patients who developed acute kidney injury were significantly higher than that of patients without AKI (p<0.001, Table 1). The changes in creatinine levels of patients without AKI was not statistically significant (p>0.05). Serum creatinine levels of patients with AKI measured at 24, 48 and 72 hours were

significantly higher than the preoperative creatinine levels (p<0.05).

Postoperative urinary NGAL concentrations of patients who developed acute kidney injury were significantly higher than that of patients without AKI (p<0.001, Table 2). The cut off value for urinary NGAL concentrations at 4 hours was found as 25.5, and considering this cut-off value the sensitivity was 81.82%, specificity was 94.12% and AUC was 0.947 (Figure 1). Gender, presence of diabetes and hypertension and surgery type were similar in patients with and without AKI (p>0.05, Table 3). There was no significant relationship between gender and AKI (X^2=0.197; p=0.493). While 6 (35.3%) of the patients who did not develop AKI were females, 11 (64.7%) of them were males; and 3 (27.3%) of the patients with AKI were females and 8 (72.7%) of them were males. There was no significant relationship between hypertension and surgery type and AKI (X^2=0.015; p=0.624, X^2=1.547; p=0.818, respectively).

CPB duration in patients with AKI was significantly higher than that of patients without AKI (111.9 minutes versus 82.7 minutes, p=0.036). The cross clamp duration of patients with AKI was significantly higher than that of patients without AKI (76.9 min versus 59.1 min, p=0.045).
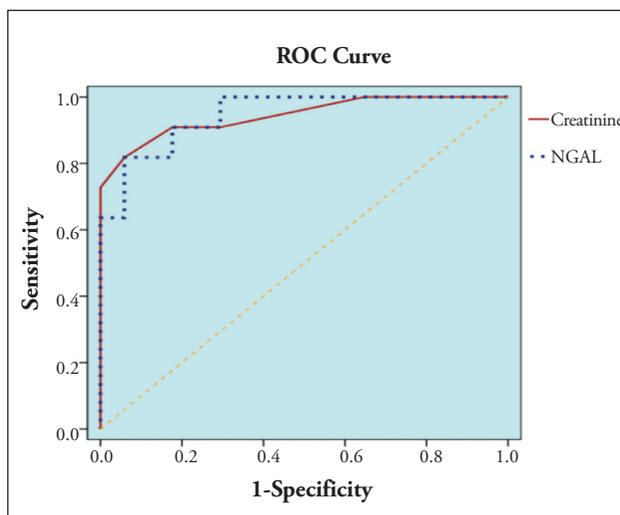

Figure 1. ROC curve showing the urinary NGAL levels at postoperative 4 hours

Table 1. Serum creatinine levels at measurement time points in patients with and without acute kidney injury (mean±SD)

	Patients without acute kidney injury	Patients with acute kidney injury	p
T1	0.8±0.1	0.9±0.1	0.133
T2	0.8±0.2	1.5±0.3	<0.001
T3	0.8±0.2	1.3±0.5	<0.001
T4	0.8±0.1	1.4±1.0	<0.001

Spearman Correlation Analysis; T1: preoperative period, serum creatinine levels before induction; T2: serum creatinine levels at postoperative 24 hours; T3: serum creatinine levels at postoperative 48 hours; T4: serum creatinine levels at postoperative 72 hours

Table 2. Urinary NGAL levels in patients with and without acute kidney injury (mean±SD)

	Patients without acute kidney injury	Patients with acute kidney injury	p
T1	7.5±6.8	19.3±38.9	0.760
T2	11.8±13.0	104.0±70.1	<0.000

Spearman correlation analysis; T1: preoperative period, urinary NGAL levels before induction; T2: urinary NGAL levels at postoperative 4 hours; SD: standard deviation

Table 3. Distribution of study variables in patients with and without acute kidney injury

		Patients without acute kidney injury		Patients with acute kidney injury		
		n	%	n	%	p
Gender	Female	6	35.3	3	27.3	X^2=0.197
	Male	11	64.7	8	72.7	p=0.493
DM	Present	5	29.4	2	18.2	X^2=0.449
	Absent	12	70.6	9	81.8	p=0.419
HT	Present	12	70.6	8	72.7	X^2=0.015
	Absent	5	29.4	3	27.3	p=0.624
Surgery	CABG	12	70.6	9	81.8	
	CABG and Valve	1	5.9	0	0.0	X^2=1.547
	Valve	2	11.8	1	9.1	p=0.818
	Valve and Aorta	1	5.9	0	0.0	
	CABG and Valve	1	5.9	1	9.1	

Chi-square test, Fisher Exact test; DM: diabetes mellitus; HT: hypertension; CABG: coronary artery bypass grafting

Intubation duration of patients without AKI (6.4 hours) was significantly shorter than that of patients with AKI (9.0 hours) (p=0.038). Length of ward stay was significantly longer in patients with AKI in comparison to that of patients without AKI (5.9 days versus 4.8 days, p=0.045).

Discussion

This present study demonstrated that urine NGAL concentrations shows a remarkable increase compared to serum creatinine levels in identifying AKI in the early postoperative period following heart surgery.

Acute kidney injury is a frequent complication encountered in open heart surgeries under CPB (13, 14). Inflammation, renal hypoperfusion and reperfusion play an important role in the development of AKI, and increase in morbidity and mortality after cardiac surgery (15).

Currently, the most frequently used marker in evaluating renal functions is serum creatinine, but it is not kidney-specific, and its levels may change in various extra-renal conditions. Other than serum creatinine, creatinine clearance, urine output and blood urea nitrogen levels are also used in identifying AKI. However, serum creatinine levels do not rise until half of the renal functions are lost; therefore it is an inadequate marker to detect AKI in the early period.

In recent years, various biomarkers have been studied to detect tubular injury in the early period with a good specificity and sensitivity (16, 17). IL-18, Cystatin C, NGAL and Kidney Injury Molecule-1 are among these biomarkers. It has been demonstrated that NGAL levels increase in intensive care unit patients after heart surgery, in sepsis, in patients undergoing percutaneous coronary interventions, in those with multiple organ failure and after renal transplantation (18, 19). A marker that can be used in AKI diagnosis should be non-invasive, reliable, rapid and easy to apply in the clinical setting, as well as should show strong marker characteristics in the receiver-operating characteristics curve (ROC). NGAL has an ideal sensitivity and specificity; and it is correlated with disease severity. Even in the absence of clinical findings consistent with AKI, it defines the level of injury. A meta-analysis reported that the best predictive performance of urinary NGAL levels is obtained in AKI cases with contrast nephropathy (20).

Koyner and colleagues (21) reported that 426 (34.9%) of 1219 patients undergoing heart surgery developed AKI. The length of intensive care and hospital stay and mortality was found to be higher in the AKI group. The rate of AKI (39.3%) found in the present study was lower than 50% rate reported by Che et al. (22) Another clinical study reported the rate of AKI as 45%, and the rate of those that required dialysis as 1.7% after heart surgery (23). In the present study, there was no difference between the patients with and without AKI in terms of gender, presence of hypertension and surgery type.

Cardiopulmonary bypass duration of above 100 minutes increases the probability of developing AKI in the postoperative period (7). Besides, aortic cross clamp duration of above 65 minutes increases the probability of developing AKI in the postoperative period (22). Prolonged intubation during CPB is a factor easing the development of AKI in the postoperative period (7, 22). In the present study as well the intubation duration of patients who developed AKI was found to be 2.6 hours longer than that of patients without AKI.

In recent years, various biomarkers have been developed to detect early renal tubular injury with a good specificity and sensitivity. Among these, the most frequently studied and most important biomarker is NGAL (16, 17). Munir et al. (7) in their study found that while serum creatinine levels were increased at 24-48 hours after surgery, urine NGAL concentrations showed a significant increase at postoperative 4 hours. Considering the cut-off value of 100 ng mL^{-1}, sensitivity was 91%, and specificity was 91%. Similar to adult cases, urinary NGAL levels give valuable information regarding AKI in paediatric cases. Mishra and colleagues (24) demonstrated that urine NGAL concentration at 2 hours after CPB is the strongest independent marker of AKI. In the study of Che and colleagues (22) for evaluating the development of AKI in patients undergoing elective cardiac surgery, according to urine NGAL concentrations at 4 hours, the sensitivity was 86% and the specificity was 67%. It was determined that urinary NGAL level measured at postoperative 4 hours in patients who were operated on with cardiopulmonary bypass; is a strong marker showing that acute kidney injury would develop in the late period (24-72 hours). Morbidity and mortality is high in AKI following CPB surgery. Therefore, rapid diagnosis is a need in these cases. Urinary NGAL levels giving information regarding AKI in a very early period such as 1-3 hours after surgery in both adult and paediatric cases shows promise about early diagnosis and treatment. There is a positive relation between CPB and aortic cross clamp duration and AKI; as the durations prolong the risk of developing AKI, the severity of AKI, and morbidity and mortality rates increase. We are in the opinion that a coherent teamwork and continued training is essential in order to shorten the CPB and aortic cross clamp durations. Enhancing the specificity by the use of various markers together, standardization of cut-off values according to markers and methods may increase the chance of success and may decrease the need for dialysis.

The major limitation of the present study is the insufficient sample size. Outcome analysis of larger patient series is needed.

Conclusion

Although larger case series are needed, we are in the opinion that AKI incidence and diagnostic cut-off level for urinary NGAL should be determined. We suggest that urinary NGAL level at postoperative 4 hours is a marker with practical benefit for AKI diagnosis that can be used in the clinic.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Bilim University Medical School.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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