ORIGINAL ARTICLE

Rapid Detection of Acute Kidney Injury by Urinary Neutrophil Gelatinase-Associated Lipocalin After Cardiopulmonary Bypass Surgery

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ABSTRACT

Objective: To determine the accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in early detection of acute kidney injury (AKI) after cardiopulmonary bypass (CPB) surgery by comparing with serum creatinine.

Study Design: Descriptive study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, AFIP in collaboration with AFIC/NIHD, Rawalpindi, from April to December 2011.

Methodology: Eighty eight patients undergoing CPB surgery in AFIC/NIHD were included by consecutive sampling. Blood samples of subjects for serum creatinine analysis were drawn pre-operatively, 4 h, 24 h and 48 h after CPB surgery. Spot urine samples for NGAL were collected at 4 h after CPB surgery. Urine samples were analyzed on Abbott ARCHITECT i2000SR analyzer whereas serum creatinine samples were measured on Beckman UniCel® DxC 600 Synchron® Clinical System.

Results: Out of 88 patients, 11 (13%) cases developed AKI 4 h postoperatively. Urinary NGAL increased markedly at 4 h postoperatively as compared to serum creatinine which showed rise at 24 – 48 h after cardiac surgery. Analysis of urine NGAL at a cutoff value of 87 ng/ml showed area under the curve of 0.91 [95% confidence interval (CI) 0.83 – 0.96] with sensitivity of 90.9% (95% CI 58.7 – 98.5) and specificity of 98.7% (95% CI 92.9-99.8). There was a positive correlation of 4 h urine NGAL and serum delta creatinine at 48 h, which was statistically significant (r_s = 0.33, p = 0.001).

Conclusion: The study demonstrated that levels of urine NGAL in patients suffering from AKI increased significantly at 4 h as compared to serum creatinine levels. Urine NGAL is an early predictive biomarker of AKI after CPB.

Key words: *Acute kidney injury. Urinary neutrophil gelatinase-associated lipocalin. Cardiopulmonary bypass surgery.*

INTRODUCTION

Cardiopulmonary bypass (CPB) surgery in adults is undertaken in more than a million patients all over the world.[1] Among them, 30% of patients suffer from a serious complication of acute kidney injury (AKI). AKI requiring dialysis occurs in upto 1% of these cases.[2] Morbidity and mortality increase with delay in diagnosing the condition.[3] Timely intervention is only possible with early diagnosis of AKI by means of effective biomarkers which can prevent progression to further renal injury. Presently serial serum creatinine measurement is taken as gold standard for identification and classification of AKI, which is not reliable during early phase of kidney dysfunction.[4]

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Neutrophil gelatinase-associated lipocalin (NGAL) was initially found in activated neutrophils. It is a potential marker for AKI, which has been identified with the help of genomic and protein micro-array technology. It is normally expressed in low concentration and is significantly increased in case of epithelial damage.[5] Urine NGAL is of excellent value in identifying patients suffering from AKI. There was a significant increase in urinary NGAL values in patients suffering from AKI as compared to non-AKI patients.[6] Levels of urine NGAL increased 24 – 48 h early as compared to serum creatinine.[7] The concentration of NGAL increased by approximately 15-fold at 2 h after CPB, which was increased to 25-fold at 4 h. Four h urine NGAL measurement following cardiac surgery showed an area under curve (AUC) of 0.96, sensitivity of 0.91 and specificity of 0.91 for prediction of AKI using a cutoff value of 100 ng/mL.[1] So, urine NGAL is considered superior to serum creatinine especially in patients arriving in intensive care unit.[8] But there is no consensus on NGAL cut-off for use in clinical settings. For establishing appropriate cutoffs for detection of AKI in various scenarios and study population, more work is required.[9]

The aim of this study was to determine the accuracy of urinary NGAL levels following cardiac bypass (CPB)

surgery to establish severity of renal impairment as compared to serum creatinine levels and to determine the appropriate cutoff post-CPB.

METHODOLOGY

This study was conducted at the Department of Chemical Pathology and Endocrinology, AFIP in collaboration with AFIC / NIHD, Rawalpindi from April to December 2011 after approval of the institutional review committee. A total number of 88 patients, scheduled for cardiovascular surgery, undergoing cardiopulmonary bypass in AFIC/NIHD, of the age 18 to 80 years were consecutively included. These patients were elaborately apprised about the study to obtain their informed consent. History-taking, physical examination findings and baseline routine investigations were carried out at the start of the study. Patients with chronic kidney disease, kidney transplant and those who were using potentially nephrotoxic drugs were excluded.

Spot urine samples were collected aseptically in plane tubes at 4 h after CPB surgery whereas blood samples of subjects were drawn aseptically in plain serum tubes pre-operatively, 4 h, 24 h and 48 h after CPB surgery. Blood and urine samples were centrifuged. Separated serum and clarified urine specimens were stored at -80°C until assayed.

Laboratory investigations of all patients were performed at AFIP. Urine samples were analyzed on ARCHITECT i2000SR analyzer (Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IIlinois, USA) by ARCHITECT urine NGAL kit which utilizes a two step immunoassay for the quantitative detection of NGAL in human urine using chemiluminescent microparticle immunoassay (CMIA) technology. Serum creatinine samples were analyzed on Beckman UniCel® DxC 600 Synchron® Clinical System (Beckman Coulter Inc., Fullerton, CA, USA) by Synchron assay which utilizes modified rate Jaffe assay.

Diagnostic criteria of AKI was established in case of an abrupt (within 48 h) absolute increase in the serum creatinine concentration of ≥ 0.3 mg/dL (26.4 µmol/L) from baseline, or a percentage increase in the serum creatinine concentration of ≥ 50 percent, or oliguria of less than 0.5 ml/kg per hour for more than 6 h.[10]

Statistical analysis of all the data was entered in Statistical Package for Social Sciences version 16 (SPSS Inc, Chicago, IL, USA). Median and inter quartile ranges (IQRs 25 – 75%) were calculated for quantitative variables. Mann-Whitney U-test was used for quantitative variables. NGAL and delta creatinine relationship were assessed by Spearman rank order correlation analysis. Urine NGAL ability for detecting AKI was assessed using area under the curve of the receiver operating characteristic (AUC-ROC) with optimal sensitivity and specificity using MedCalc statistical package. P-value of < 0.05 was considered statistically significant.

Table I: Characteristics of CPB patients without AKI as compared with the patients who developed AKI within 48 h.

Parameter	No AKI (n = 77) Median (IQR)	AKI (n = 11) Median (IQR)	p-value
Age (years)	51 (45-61)	56 (47-64)	0.325
CPB time (minutes)	90 (60-105)	100 (92-126)	0.033
AXC time (minutes)	55 (37-66)	65 (53-80)	0.025
Pre-operative eGFR (ml/min/1.73 m^2)	81 (67-94)	71 (56-85)	0.087
Baseline serum creatinine (umol/L)	88 (77-98)	95 (80-112)	0.133
Serum creatinine 4 h (umol/L)	85 (71-96)	87 (74-111)	0.614
Serum creatinine 24 h (umol/L)	88 (76-102)	108 (78-123)	0.038
Serum creatinine 48 h (umol/L)	91 (79-106)	134 (123-164)	< 0.001
Delta creatinine (%)	6 (0-22)	46 (32-58)	< 0.001
Urinary NGAL (ng/ml)	5 (2-15)	180 (105-277)	< 0.001

CPB = Cardiopulmonary bypass; AKI = Acute kidney injury ; IQR = Interquartile range; TVCAD = Triple vessel coronary artery disease; CABG = Coronary artery bypass grafting; AXC = Aortic cross clamp; eGFR = Estimated glomerular filtration rate; NGAL = Neutrophil gelatinase-associated lipocalin.

RESULTS

A total of 88 patients undergoing CPB, who met the inclusion and exclusion criteria, were consecutively included. There were 76 males (86%) and 12 females (14%) ranged from 18 to 80 years, with mean age of 52 years and mean weight of 67 kilograms (kg). Patient characteristics of the study are shown in Table I.

Based on acute kidney injury network (AKIN) criteria, subjects were grouped into AKI and those with no AKI. Age, estimated creatinine clearance, serum creatinine levels pre-operatively and at 4 h did not show any significant difference between two groups. Whereas serum creatinine levels at 24 h, 48 h and urinary NGAL levels at 4 h showed significant difference between two groups. CPB time and aortic cross clamp (AXC) time was increased in AKI group as compared to group with no AKI. Triple vessel coronary artery disease (TVCAD) was present in 73% cases and similarly, the occurrence of AKI was found more common in this disease. Coronary artery bypass grafting was performed on 84% cases.

Eleven patients (13%) developed AKI fulfilling the criteria of AKIN.[10] Urine NGAL ability was assessed to predict AKI after cardiac bypass. NGAL levels in AKI group of 180 ng/ml (IQR: 105 – 277 ng/ml) at 4 h were significantly higher than non-AKI group of 5 ng/ml (IQR: 2 – 15 ng/ml). Serum creatinine showed significant rise with delay of 24 – 48 h in AKI group. Urine NGAL ability to predict clinical outcome was assessed using Spearman rank order correlation analysis. There was a positive correlation between 4 h urine NGAL and delta creatinine at 48 h, which was statistically significant ($r_s = 0.33$, $p = 0.001$).

ROC curves for urine NGAL were generated, AUCs calculated at 4 h after cardiopulmonary bypass surgery,

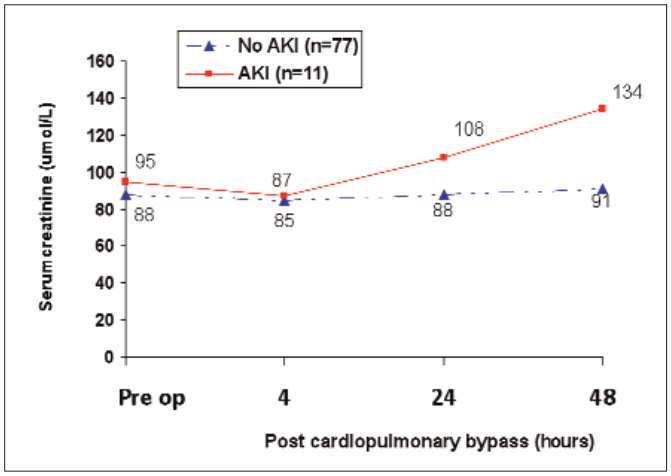
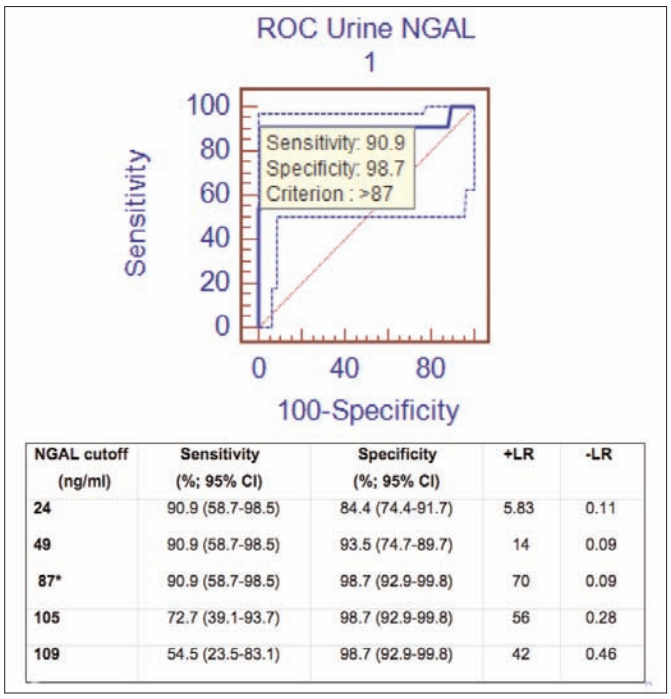


Figure 1: Serum creatinine measurements obtained at various time point pre- and post-CPB showing AKI vs. no AKI.



NGAL cutoff (ng/ml)	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	+LR	-LR
24	90.9 (58.7-98.5)	84.4 (74.4-91.7)	5.83	0.11
49	90.9 (58.7-98.5)	93.5 (74.7-89.7)	14	0.09
87*	90.9 (58.7-98.5)	98.7 (92.9-99.8)	70	0.09
105	72.7 (39.1-93.7)	98.7 (92.9-99.8)	56	0.28
109	54.5 (23.5-83.1)	98.7 (92.9-99.8)	42	0.46

Figure 2: Receiver operating characteristic (ROC) curve of urine NGAL levels at 4 h after CPB for predication of AKI and table shows sensitivity, specificity, positive and negative predictive value of urine NGAL cutoffs at 4 h post-CPB. CI, confidence interval; +LR, positive likelihood ratio; -LR, negative likelihood ratio; * Urine NGAL optimal cutoff.

sensitivities, specificities and predictive values at different cutoff concentrations are listed (Figure 2). For urine NGAL, a cutoff of 87 ng/ml yielded sensitivity of 90.9% [95% confidence interval (CI) 58.7 – 98.5] and specificity of 98.7% (95% CI 92.9 – 99.8) at 4 h after CPB surgery with AUC of 0.91(95% CI 0.83 – 0.96) for predication of AKI, which was representative of an excellent biomarker.

DISCUSSION

AKI, also known as acute renal failure (ARF), is one of the frequent complications of cardiac bypass surgery. Lack of availability of suitable biomarkers for depicting kidney injury hindered in timely preventive and therapeutic measures. Inflammation, renal hypoperfusion and reperfusion were considered causative factors for initiation of kidney injury, increasing mortality and morbidity significantly after cardiac surgery.[11]

Serum creatinine, which is now considered as an inadequate marker, is most frequently used for assessing renal functions, as serum creatinine levels rise when more than 50% of kidney functions are lost. Serum creatinine is not specific for kidney injury. Levels can vary widely depending on non-renal factors such as gender, muscle mass and hydration status. Apart from serum creatinine, creatinine clearance, urine output and blood urea nitrogen levels were also used for establishing AKI. In recent years, several biochemical markers of tubular damage with good sensitivity and specificity were detected to fulfill this diagnostic gap.[12] These biomarkers include IL-18, cystatin C, NGAL and kidney injury molecule-1 (KIM-1).[13-15]

NGAL, a 25 kDa protein has been established to rise considerably in patients with AKI but not in the corresponding controls.[16] There are many ongoing studies depicting similar relationship in cardiac surgery, critically ill patients presenting in the intensive care units, sepsis, percutaneous coronary interventions, multi-organ failure and renal transplantation.[17-19] Urine NGAL was established as an excellent biomarker in detecting AKI post-CPB. A single measurement of urinary NGAL helps to distinguish AKI from normal function.

NGAL fulfilling the criteria of ideal biomarker is sensitive and specific, correlate with severity and quantitatively describe the level of injury even in the absence of typical clinical signs. The occurrence of AKI was earlier revealed for urine NGAL by Wagener and colleagues.[11] Whereas association of duration and severity of AKI with NGAL has also been found in children undergoing cardiac surgery.[1] In this study, a rapid rise of urine NGAL was observed within 4 h as compared to serum creatinine post-CPB, depicting occurrence and severity of renal injury. Similarly, patients in whom cardiac bypass time was greater than 100 minutes and aortic cross clamp time was greater than 65 minutes were more prone for developing AKI. A decrease in serum creatinine, immediately after bypass surgery, was noticed in most of the patients with AKI and no AKI due to plenty of fluid infusion. Whereas, NGAL levels rose immediately after operation in AKI patients (n = 11) when serum creatinine had not yet changed from baseline.

This study has several strengths. First, patients were recruited who had renal injury during cardiac bypass having normal kidney functions before the surgery. Secondly, collection of urine samples were of non-invasive nature and easy to collect.

The limitations of this study comprise serum creatinine being used for diagnostic criteria in AKI, which itself is a

poor marker of kidney dysfunction, difficulty in obtaining samples from patients with severe oliguria, potential changes in urinary biomarker concentration induced by the overall fluid status and co-morbid conditions in adults.

Clinical utility of urine NGAL include avoidance of nephrotoxic drug use,[20] and a more meticulous monitoring of blood pressure and urine output for maintaining adequate renal perfusion post-CPB. Findings of this study need to be validated on patients with pre-existing renal dysfunction especially those suffering from chronic kidney disease.

CONCLUSION

This study demonstrated that urine NGAL levels rose significantly in patients fulfilling the criteria for AKI much earlier as compared to serum creatinine levels in early hours after cardiac surgery. So, urine NGAL is an early predictive biomarker of AKI after CPB.

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