Research Article

Early Detection of Acute Kidney Injury by Measuring Urinary Neutrophil Gelatinase Associated Lipocalin After Cardiopulmonary Bypass in Cardiac Surgery

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Abstract

Background: Acute kidney injury (AKI) is a common complication after cardiac surgery and increases morbidity and mortality. The identification of reliable biomarkers that allow earlier diagnosis of AKI in the postoperative period may increase the success of therapeutic interventions. Neutrophil gelatinase-associated lipocalin (NGAL) may be an early biomarker for cardiac surgery-associated (CSA) AKI. **Objective:** We investigated whether increased urinary NGAL concentrations were predictive of AKI within the early postoperative in-hospital stay.**Patients and Methods:** We conducted a prospective observational study involving ١٠٠ consecutive adults patients undergoing cardiac surgery. Patients with pre-existing chronic renal failure requiring dialysis were excluded. Urinary NGAL was measured from urinary samples obtained preoperatively and two-hours after surgery (Quantikine ELISA, Human Lipocalin-٢/NGAL Immunoassay). Plasma creatinine level was determined preoperatively and daily after surgery. AKI was defined as an increase in serum creatinine in the postoperative period by more than ٠.٥ mg/dL (٠.٤٤ mmol/L) from baseline, as defined by ٢٠٠٣ Acute Renal Failure Consensus Conference. The potential role of NGAL in predicting postoperative AKI was analyzed with a logistic regression model.**Results:** Patients with AKI showed a significant increase in total CPB time and cross-clamp time. The significant changes from baseline in uNGAL appear within ٢ hours after surgery in patients with AKI while the significant changes in other renal function tests start within ٢٤-٤٨ hours and remain significant till the ٥th day after surgety. Using Receiver operating characteristic (ROC) curve revealed a large significant area under curve (AUC) for postoperative uNGAL [٠.٩٧ for Egyptian patients (Confidence interval: ٠.٩٢-١) and ٠.٩٥ for Italian patients (Confidence interval: ٠.٨٩-١)]. Thus, uNGAL is a potent predictive test for early detection of postoperative AKI.A threshold of ٢.٥ ng/dL (٢٥٠ ng/mL) for uNGAL on ROC curve yielded sensitivity from ٨٠-٩٣٪ and specificity from ٨٠-١٠٠٪. **Conclusion:** Urinary NGAL is a biomarker for very early risk stratification of AKI after cardiac surgery and may be useful as a basis for early interventional strategies to prevent CSA-AKI. It was demonstrated to be an optimal predictor of postoperative AKI on patients who undergo cardiac surgery using cardiopulmonary bypass.

Keywords: Acute kidney injury (AKI), Cardiac surgery,Biomarkers,Neutrophil gelatinase-associatedlipocalin(NGAL).

Introduction

Acute kidney injury (AKI) occurs frequently, complicating ٣٠–٤٠٪ of adult and pediatric cardiac surgeries[١,٢]. Early prediction of postoperative renal injury could help practitioners better evaluate the risk of AKI and to initiate early interventional or precautionary measures[٣,٤]. In recent years, several novel human biomarkers have been demonstrated to detect acute tubular injury and have shown promise in their ability to precede and/or complement serum creatinine in the diagnosis of AKI[٥,٦]. Several urinary proteins have been shown to serve as biomarkers of AKI after

cardiac surgery, including neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CyC), kidney injury molecule-١ (KIM-١), interleukin-١٨ (IL-١٨), and α-glutathione-S-transferase (α-GST), however limited data are available comparing the ability of these markers to predict renal outcomes at the time of AKI diagnosis [٧].

The aim of the present study was to evaluate the use of urinary Neutrophil Gelatinase Associated Lipocalin (uNGAL) as early predictor of Cardiac Surgery Associated Acute Kidney Injury (CSA-AKI) in adults undergoing Cardio-Pulmonary Bypass for cardiac surgery.

Patients and Methods

A prospective comparative multicenter observational study was conducted in two settings at cardiovascular and thoracic surgery departments in:Egypt: Ain Shams University Hospital, Cairo and El-Minia University Hospital, El-Minia; and Italy: Santa Croce Hospital, Cuneo.The study included ١٠٠ adult patients (٦٠ from Egypt and ٤٠ from Italy) undergoing cardiac surgery using CPB, between ٢٠١٠ and ٢٠١٣. Written informed consent was obtained from all subjects. Patients with the following criteria were excluded: pre-existing renal failure requiring regular dialysis and off pump cardiac surgery.

Patients were classified in four groups: group A: ٣٠ patients from Egypt with no AKI; group B: ٣٠ patients from Egypt with postoperative AKI; group C: ١٠ patients from Italy with no AKI; and group D: ٣٠ patients from Italy with postoperative AKI.

AKI was defined as an increase in serum creatinine in the postoperative period by more than ٠.٥ mg/dL (٠.٤٤ mmol/L) from baseline as defined by ٢٠٠٣ Acute Renal Failure Consensus Conference[٨].

Data collection:

Demographic Data, History of Systemic diseases as diabetes mellitus (DM), Systemic hypertension (HTN), Peripheral vascular diseases (PVD), Chest diseases, liver impairment and Renal impairment.Cardiac condition as Left ventricle Ejection Fraction (EF), pre-operative Congestive heart failure, Pre-operative Cardiogenic shock and use of pre-operative IABP, left main coronary disease. Operative Data as Status (Elective, Urgent, Emergent or salvage) and Procedure performed. Perfusion Data as Cardio Pulmonary Bypass (CPB) time, Cross-clamp time, Pressure during CPB (minimum and maximum), Hemolysis, Hemodilution (Hematocrit level) , Urine output, Balance and Method of weaning of bypass either without support, on minimal or high dose pharmacological support, or on Intra Aortic Balloon Pump (IABP) support.

Sample Collection and Measurements:

Urine was collected via a urinary catheter preoperatively and ٢ hours after completion of surgery. Urine was centrifuged at ١٠٠٠g for ١٥ minutes to remove any debris. Samples were stored at – ٢٠ C for a maximum of ١٢ months and then thawed once for assay. Urinary Neutrophil Gelatinase–Associated Lipocalin (uNGAL) was measured by ELISA (Enzyme linked Immuno-Sorbant Assay). The kits used are Quantikine ELISA, Human Lipocalin-٢/NGAL Immunoassay. Blood Urea, Serum Creatinine and estimated Criatinine Clearance were evaluated pre and post-operative till ٥th Post-operative day. Estimated Creatinine Clearance (e Cr CL) was calculated by CockcroftGault (C&G) formula [٩].

Statistical Analysis

Analysis of data was done by IBM computer using Statistical Package for Social Science (SPSS) version ٢٠.٠. The quantitative variables were described as: mean ± standard deviation (SD), and the qualitative variables were described as number and percentage. Chi-square test was used to compare qualitative variables between groups. Paired t-test was used to compare dependant normally distributed variables. Unpaired t-test was used to compare quantitative variables in parametric data (SD <٥٠٪ mean). Mann Whitney Willcoxon U test was used instead of unpaired t-test in non-parametric data (SD >٥٠٪ mean). Pearson's correlation was done to test for linear relations and Correlation co-efficient test (r-value) was used to rank different variables either positively or inversely.

Receiver-Operating Characteristic (ROC) curve was used to test the efficiency of different diagnostic tests including uNGAL in predicting acute kidney injury. In a ROC curve the true positive rate (sensitivity) is plotted in function

of the false positive rate (١-Specificity) for different cut-off points of a parameter.The area under the ROC curve (AUC) is recognized as the measure of a diagnostic test's discriminatory power. P value <٠.٠٥ was considered statistically significant.

Results

Analysis of Preoperative data (Table ١) revealed a mild significant difference in mean LVEF between group A and B, but both means were within normal range (٥٩±٥ in group A versus ٥٥±٨ in group B, P=٠.٠٣). There was no significant difference in frequency of CHF and left main disease between both groups A & B.Also between group C and group D there was no significant difference in mean of EF, frequency of CHF and left main disease.

Analysis of Operative data (Table ١) revealed that: Group B showed a significant increase in total CPB time (٩٤.٨٠±٤١.٥٤ min versus ٧١.٩٠±٣٧.٧١ min), cross-clamp time (٦١.٤٣±٣٠.٨٠ min versus ٤٥.٦٦±٢٤.٧٧ min), urine output (٩٧١.٦±٧٦٣.٨ mL versus ٥٣٩.٣±٣٢٣ mL) and weaning of bypass using minimal dose pharmacological support (٦٦.٧٪ versus ١٣.٣٪) when compared to group A. Also group D showed a significant increase in total CPB time (٩٤.٨٠±٤١.٥٤ min versus ٧١.٩٠±٣٧.٧١ min) and cross-clamp time (٦١.٤٣±٣٠.٨٠ min versus ٤٥.٦٦±٢٤.٧٧ min) when compared to group C. Analysis of renal function tests and uNGAL (Table ٢) revealed that: The mean values of S.Cr. and BUN were significantly higher and the mean values of Cr.Cl were significantly lower in group B when compared to group A from ١st to ٥th day after surgery. The mean values of S.Cr. and BUN were significantly higher and the mean values of Cr.Cl were significantly lower in group D when compared to group C from ١st to ٥th day after surgery. There was no significant difference in preoperative uNGAL while postoperative uNGAL was significantly higher in group B (٧.٣٧±٤.٥٩ ng/dL) than group A (٠.٩٣±٠.٥٠ ng/dL). There was no significant difference in preoperative uNGAL while postoperative uNGAL was significantly higher in group D (١٢.٠٣±٧.١٥ng/dL) than group C (١.٧٠±١.٢٢ng/dL). The significant changes from baseline in uNGAL appear within ٢ hours after surgery in patients with AKI while the significant changes in other renal function tests start within ٢٤-٤٨ hours and remain significant till the ٥th day after surgery.

Correlation Analysis between postoperative eu NGAL and Renal function tests:

There was no significant correlation between postoperative uNGAL and renal function tests from ١st to ٥th day after surgery in group A & C (Table ٣). While in group B, postoperative uNGAL showed a significant correlation with ١st day S.Cr. (r-value=٠.٣٧, p-value=٠.٠٤), ٢nd day S.Cr. (r-value=٠.٤٠, p-value=٠.٠٢), ٣rd day S.Cr. (r-value=٠.٤٦, p-value=٠.٠١), ٤th day S.Cr. (r-value=٠.٥٩, p-value=٠.٠٠١), ٥th day S.Cr. (r-value=٠.٤٠, p-value=٠.٠٢), ٤th day BUN (r-value=-٠.٣٨, p-value=٠.٠٣), ٥th day BUN (r-value=٠.٦٥, p-value=٠.٠٠٠١), ٤th day Cr.Cl (r-value=٠.٤٦, p-value=٠.٠١), and ٥th day Cr.Cl (r-value=-٠.٤٨, p-value=٠.٠٠٧). While group D, postoperative uNGAL showed a significant correlation with ١st day Cr.Cl (r-value=-٠.٦٧, p-value=٠.٠٣), ٢nd day Cr.Cl (r-value=-٠.٦٤, p-value=٠.٠٤) and ٣rd day Cr.Cl (r-value=-٠.٦٧, p-value=٠.٠٣). There was no significant correlation between postoperative uNGAL and other renal function tests up to ٥th day after surgery in group D.

Evaluation of predictive value of Predictors of AKI:

The receiver operating characteristics (ROC) curve was used to determine the predictive value for the evaluated predictors of CSA AKI in group B and D, through illustrating the relation between sensitivity (True positive) and ١-specificity (False positive) for preoperative EF, preoperative serum Cr., preoperative BUN, preoperative uNGAL, and postoperative uNGAL (Figs. ١,٢).

In group B the area under the curve (AUC) was ٠.٦٢ for EF, ٠.٥٣ for serum Cr., ٠.٥٦ for BUN, ٠.٥٧ for preoperative uNGAL, and ٠.٩٧ for postoperative uNGAL. The only significant AUC was observed with postoperative uNGAL (P=٠.٠٠٠١), which indicates a significant high predictive value of postoperative uNGAL than other evaluated predictors of AKI (Table ٤). In group D the area under the curve (AUC) was ٠.٥٦ for EF, ٠.٦٩ for serum Cr., ٠.٦٤ for BUN, ٠.٦٢ for preoperative uNGAL, and ٠.٩٥ for postoperative uNGAL. The only significant AUC was observed with postoperative uNGAL (P=٠.٠٠٠١), which indicates a significant high

predictive value of postoperative uNGAL than other evaluated predictors of AKI (Table ٤).Multiple cut-off points of postoperative uNGAL

for detection of AKI determined on ROC curves (Table ٥). The best cut-off was ١٩٠ ng/mL which yielded sensitivity of ٩٦٪ and specificity of ٩٧٪ in group B, and yielded sensitivity of ٩٦٪ and specificity of ٧٠% in group D.

Table (١): Demographic, preoperative and operative data of the studied groups

Variables	Group A (n=٣٠)	Group B (n=٣٠)	P-value A & B	Group C (n=١٠)	Group D (n=٣٠)	P-value C & D
Age (years)	٣٩.١٦±١٣.٨١	٤٥.٠٣±١٢.٣٣	٠.٠٨	٧٠±٦.٩١	٧٥.٥٠±٨.٠٢	٠.٠٦
Sex M F	١٤(٤٦.٧٪) ١٦(٥٣.٣٪)	١٨(٦٠٪) ١٢(٤٠٪)	٠.٣٠	٦(٦٠٪) ٤(٤٠٪)	٢١(٧٠٪) ٩(٣٠٪)	٠.٥٥
Weight (kg)	٧٢.٦٠±١٥.٣٩	٧٥.٦٠±١٣.١١	٠.٤٢	٧١.٣٠±١٧.٣٢	٧٥±١٢.٧٧	٠.٤٧
Height (m)	١.٦٥±٠.١٠	١.٦٩±٠.٠٨	٠.١٥	١.٦٥±٠.٠٩	١.٦٦±٠.١٠	٠.٨٢
BMI (kg/m^2)	٢٦.٢±٤	٢٦.٣±٣.٨	٠.٩٦	٢٥.٦±٤.٨	٢٧±٤.٥	٠.٤٠
Risk factors						
Smoking	٩(٣٠٪)	١٠(٣٣.٣٪)	٠.٧٨	٢(٢٠٪)	٩(٣٠٪)	٠.٥٤
DM	٤(١٣.٣٪)	١٠(٣٣.٣٪)	٠.٠٦	٤(٤٠٪)	١١(٣٦.٧٪)	٠.٨٥
Insulin therapy	٢(٦.٧٪)	٤(١٣.٣٪)	٠.٧٣	١(١٠٪)	٥(١٦.٧٪)	٠.٤٧
HTN	٦(٢٠٪)	١٢(٤٠٪)	٠.٠٩	٩(٩٠٪)	٢٤(٨٠٪)	٠.٤٧
PVD	٠(٠٪)	٠(٠٪)	NA	٠(٠٪)	٤(١٣.٣٪)	٠.٢٢
COPD	٠(٠٪)	١(٣.٣٪)	٠.٣١	١(١٠٪)	٧(٢٣.٣٪)	٠.٣٦
Liver disease	٠(٠٪)	٠(٠٪)	NA	١(١٠٪)	١(٣.٣٪)	٠.٤٠
Cardiac condition						
EF (%)	٥٩±٥	٥٥±٨	٠.٠٣*	٥٣±٨	٥٠±١٢	٠.٤٣
CHF	٠(٠٪)	١(٣.٣٪)	٠.٣١	٠(٠٪)	٣(١٠٪)	٠.٢٩
Left main disease	٠(٠٪)	٠(٠٪)	NA	١(١٠٪)	٤(١٣.٣٪)	٠.٧٨
Priority:						
-Elective	٣٠(١٠٠٪)	٣٠(١٠٠٪)	NA	٨(٨٠٪)	٢٠(٦٦.٧٪)	٠.٤٢
-Urgent	٠(٠٪)	٠(٠٪)	NA	٠(٠٪)	٨(٢٦.٧٪)	٠.٠٦
-Emergent	٠(٠٪)	٠(٠٪)	NA	٢(٢٠٪)	٢(٦.٧٪)	٠.٢٢
Procedure:						
-CABG	٧(٢٣.٣٪)	١١(٣٦.٧٪)	٠.٢٥	٥(٥٠٪)	٧(٢٣.٣٪)	٠.٥١
-Valve	١٦(٥٣.٣٪)	١٥(٥٠٪)	٠.٧٩	٣(٣٠٪)	١٢(٤٠٪)	٠.٥٧
-CABG+Valve	٠(٠٪)	١(٣.٣٪)	٠.٣١	٠(٠٪)	١(٣.٣٪)	٠.٥٥
-CHD	٥(١٦.٧٪)	١(٣.٣٪)	٠.٠٨	٠(٠٪)	١(٣.٣٪)	٠.٥٥
-Redo valve	٢(٦.٧٪)	١(٣.٣٪)	٠.٥٥	١(١٠٪)	١(٣.٣٪)	٠.٤٠
-Aortic	٠(٠٪)	١(٣.٣٪)	٠.٣١	١(١٠٪)	٧(٢٣.٣٪)	٠.٣٦
-Traumatic	٠(٠٪)	٠(٠٪)	NA	٠(٠٪)	١(٣.٣٪)	٠.٥٥
Arterial canula:						
-Aortic	٣٠(١٠٠٪)	٣٠(١٠٠٪)	NA	١٠(١٠٠٪)	٢٥(٨٣.٣٪)	٠.١٦
-Femoral	٠(٠٪)	٠(٠٪)	NA	٠(٠٪)	٥(١٦.٧٪)	٠.١٦
Total CPB time (min)	٧١.٩٠±٣٧.٧١	٩٤.٨٠±٤١.٥٤	٠.٠٢*	٧٦.١٠±١١.١٦	١٢٨.١٣±٥٤.٤٥	٠.٠٠٥*
Cross-clamp time(min)	٤٥.٦٦±٢٤.٧٧	٦١.٤٣±٣٠.٨	٠.٠٣*	٦١.٩٠±١١.٠٤	٩٧.٥٨±٣٨.٦٤	٠.٠٠٧*
Minimum Pressure (mmHg)	٥١.١٣±٧.٥٧	٥١.١٠±٨.٢٩	١	٦٧±٨.٢٣	٦٤.٩٦±٩.٣١	٠.٥٤
Maximum Pressure (mmHg)	٧٦.٥٠±٦.٠٣	٧٥.٨٣±٧.٧٩	٠.٧١	٩٣.٥٠±١١.٠٦	٨٦.٦٣±١٠.١٢	٠.٠٧
Mean Hct (%)	٢٨.١٣±٢.٨٨	٢٨±٣.١٥	٠.٨٦	٣٠.٠٦±٤.١١	٢٧.٨١±٤.٤١	٠.١٦
Urine (mL)	٥٣٩.٣±٣٢٣	٩٧١.٦±٧٦٣.٨	٠.٠٠٦*	٢٥٧±١٦٩.١١	٢٦٤±٢٠٧.٩٠	٠.٩٢
Use of hemofilter	٠(٠٪)	٠(٠٪)	NA	٢(٢٠٪)	١٢(٤٠٪)	٠.٢٥
Weaning of bypass:						
-No support	٢٦(٨٦.٧٪)	٧(٢٣.٣٪)	٠.٠٠٠١*	٣(٣٠٪)	٩(٣٠٪)	١
-Minimal dose support	٤(١٣.٣٪)	٢٠(٦٦.٧٪)	٠.٠٠٠١*	٧(٧٠٪)	١٦(٥٣.٣٪)	٠.٣٥
-High dose support	٠(٠٪)	٢(٦.٧٪)	٠.١٥	٠(٠٪)	٤(١٣.٣٪)	٠.٢٢
-IABP support	٠(٠٪)	١(٣.٣٪)	٠.٣١	٠(٠٪)	١(٣.٣٪)	٠.٥٥

BMI: Body mass index. M: Male. F: Female. DM: Diabetes mellitus. HTN: Hypertension. PVD: Peripheral vascular disease. COPD: Chronic obstructive pulmonary disease. EF: Ejection fraction. CHF: Congestive heart failure. CABG: Coronary artery bypass grafting. CHD: Congenital heart disease. CPB: Cardiopulmonary bypass. IABP: Intra-aortic balloon pump. * significant difference. NA: Not appropriate

Table (٢): Pre- and post-operative renal function tests and uNGAL of the studied groups

Variables	Group A (n=٣٠)	Group B (n=٣٠)	P-value A & B	Group C (n=١٠)	Group D (n=٣٠)	P-value C & D
Baseline S.Cr. (mg/dL)	١.٠٢±٠.٣٢	١.٠٤±٠.٢٥	٠.٨٠	٠.٩٣±٠.٣٧	١.٠٨±٠.٣٥	٠.٢٤
Baseline BUN (mg/dL)	٣٥±١٤.٤٩	٣٦.٨٦±١٥.١٦	٠.٦٢	٤٦.٧٠±٢٤.١٠	٤٨.٢٠±١٢.٢٨	٠.٨٢
Baseline Cr.Cl (mL/min)	٩٦.٩٤±٣٧.٤٠	٩٣.٤٤±٢٨.٠٤	٠.٦٨	٧٤.٨٠±٢١.٧٢	٦٣.٧١±٢١.٤٩	٠.١٦
Day١S.Cr (mg/dL)	١.٠٦±٠.٢٦	١.٣٣±٠.٣١	٠.٠٠١*	٠.٨٢±٠.٢٨	١.٢٣±٠.٤٦	٠.٠١*
Day١BUN (mg/dL)	٣٩.٢٠±١١.٥٧	٤٧.٦٦±١٧.٤٧	٠.٠٣*	٤٣±١٣.٢٠	٥٥.٠٩±١٩.٦٥	٠.١١
Day١Cr.Cl (mL/min)	٩١.٠٧±٣٣.١٥	٧٢.٩١±١٩.٨٧	٠.٠١*	٨٣.٩٥±٢٥.٦٤	٥٦.٧٨±١٨.٦١	٠.٠٠١*
Day ٢ S.Cr (mg/dL)	١.١٠±٠.٢٥	١.٥٤±٠.٣٧	٠.٠٠٠١*	٠.٨٩±٠.٢٥	١.٥٩±٠.٥٣	٠.٠٠٠١*
Day ٢ BUN (mg/dL)	٤٠.٢١±١١.٣٨	٥٣.٦٦±١٧.١٥	٠.٠٠١*	٤٢.١٠±١٣.٨٧	٦٣.٢١±٢٣.٤٦	٠.٠١*
Day ٢ Cr.Cl (mL/min)	٨٨.٢٧±٣٦.٧١	٦٣.١٤±١٧.٨٣	٠.٠٠١*	٧٥.٢٣±١٩.٤٨	٤٣.٩٦±١٦.٠٣	٠.٠٠٠١*
Day ٣ S.Cr (mg/dL)	١.٠٩±٠.٢٨	١.٧٧±٠.٤٥	٠.٠٠٠١*	٠.٨٣±٠.٢٧	١.٦٩±٠.٦٦	٠.٠٠٠١*
Day ٣ BUN (mg/dL)	٤٢.١٨±١١.٦٧	٥٩.٤٦±١٧.٠٩	٠.٠٠٠١*	٤٣.٧٠±١٤.٣٩	٨٢.٠٤±٢٥.٢٦	٠.٠٠٠١*
Day ٣ Cr.Cl (mL/min)	٨٥.٣٦±٣٤.٢٣	٥٥.٢٢±١٥.١٢	٠.٠٠٠١*	٨١.٦٣±٢٠.٦١	٤٣.٥٧±١٨.٢٢	٠.٠٠٠١*
Day ٣ S.Cr (mg/dL)	١±٠.٣٠	١.٩٥±٠.٥٢	٠.٠٠٠١*	٠.٨٤±٠.٢٦	١.٦٤±٠.٦٣	٠.٠٠٠١*
Day ٤ BUN (mg/dL)	٤١.٠٣±١٧.٢٦	٦٤±١٥.٦١	٠.٠٠٠١*	٤٣.٣٠±١٦.٦٨	٨٩.٦٢±٢٧.٢٥	٠.٠٠٠١*
Day ٤ Cr.Cl (mL/min)	١٠١.٣٨±٤٧.٢٤	٥٠.٤٤±١٤.٤٠	٠.٠٠٠١*	٨٠.١٧±٢١.٤٩	٤٥.١٦±١٩.٨٣	٠.٠٠٠١*
Day ٥ S.Cr (mg/dL)	٠.٩٦±٠.٢٦	٢±٠.٦٢	٠.٠٠٠١*	٠.٧٦±٠.٢٣	١.٤٨±٠.٥٩	٠.٠٠١*
Day ٥ BUN (mg/dL)	٣٥.٧٦±١٣.٤٤	٦٦.٥٠±١٥.١٩	٠.٠٠٠١*	٣٩.٣٠±١٤.٠٣	٨٤.٦١±٢٧.٣٧	٠.٠٠٠١*
Day ٥ Cr.Cl (mL/min)	١٠٣.٢٨±٤٤.٥٦	٥٠.٣١±١٦.٠١	٠.٠٠٠١*	٨٨.٤٩±٢٢.٨٧	٤٩.٧٢±١٨.٩٠	٠.٠٠٠١*
uNGAL (ng/dL)						
Preoperative	١.١٤±١.٢٥	١.٥٠±١.٤٧	٠.٣٢	٣.٩٠±٣.٧٣	٧.٢٦±٦.٧٤	٠.١٤
Postoperative	٠.٩٣±٠.٥٠	٧.٣٧±٤.٥٩	٠.٠٠٠١*	١.٧٠±١.٢٢	١٢.٠٣±٧.١٥	٠.٠٠٠١*

uNGAL: Urinary neutrophil gelatinase–associated lipocalin. S.Cr: Serum creatinine. BUN: Blood urea nitrogen. Cr.Cl: Creatinine clearance. *significant difference

Table (٣): Correlation of postoperative uNGAL with postoperative renal function tests in the studied groups

Variables	Group A (n=٣٠)		Group B (n=٣٠)		Group C (n=١٠)		Group D (n=٣٠)	
	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
Day١S.Cr	٠.١٨٥	٠.٣٢	٠.٣٧٧	٠.٠٤*	٠.٢١٥	٠.٢٥٣	٠.٠٠٩	٠.٩٧
Day١BUN	٠.٠٧٦	٠.٦٨	٠.٢٧٧	٠.١٣	٠.٤٨١	٠.١٣٤	٠.١٩	٠.٥٨
Day١Cr.Cl	-٠.١١٦	٠.٥٤	-٠.١٩٦	٠.٢٩	-٠.٢٤٧	٠.١٨٩	-٠.٦٧	٠.٠٣*
Day ٢ S.Cr	٠.٢٣٥	٠.٢١	٠.٤٠٣	٠.٠٢*	٠.٢٢٦	٠.٢٢٩	-٠.١٠	٠.٧٦
Day ٢ BUN	٠.١٠٧	٠.٥٧	٠.٢٨٣	٠.١٣	٠.١٦٨	٠.٤٩٣	٠.S٠٢	٠.٩٥
Day ٢ Cr.Cl	-٠.١٥٤	٠.٤١	-٠.١٨٦	٠.٣٢	-٠.١٨٨	٠.٣٢١	-٠.٦٤	٠.٠٤*
Day ٣ S.Cr	٠.١٧٩	٠.٣٤	٠.٤٦٢	٠.٠١*	٠.١٤٤	٠.٤٤٨	-٠.٠٩	٠.٨٠
Day ٣ BUN	-٠.٠٢٤	٠.٩٠	٠.٢٩٣	٠.١١	٠.٠٦٥	٠.٧٥٩	-٠.٠٥	٠.٨٩
Day ٣ Cr.Cl	-٠.٢٤٤	٠.١٩	-٠.٣٠٤	٠.١٠	-٠.٠١٦	٠.٩٣٢	-٠.٦٧	٠.٠٣*
Day ٤ S.Cr	٠.٢٥٤	٠.١٧	٠.٥٩٠	٠.٠٠١*	٠.١٦٤	٠.٣٨٦	-٠.١٢	٠.٧٣
Day ٤ BUN	٠.٠٢٢	٠.٩١	٠.٤٠٨	٠.٠٢*	-٠.٠١٨	٠.٩٣٠	-٠.٢١	٠.٥٤
Day ٤ Cr.Cl	-٠.٢١٨	٠.٢٤	-٠.٣٨٠	٠.٠٣*	-٠.٠٦٢	٠.٧٤٤	-٠.٥٨	٠.٠٧
Day ٥ S.Cr	٠.١٤١	٠.٤٥	٠.٦٥٤	٠.٠٠٠١*	٠.١٧٢	٠.٣٧١	-٠.١٦	٠.٦٤
Day ٥ BUN	٠.٠٤٥	٠.٨١	٠.٤٦١	٠.٠١*	-٠.٠٦٣	٠.٧٦١	-٠.٢٥	٠.٤٨
Day ٥ Cr.Cl	-٠.١٠٥	٠.٥٨	-٠.٤٨٠	٠.٠٠٧*	-٠.١١٢	٠.٥٦٤	-٠.٥٥	٠.٠٩

S.Cr: Serum creatinine. BUN: Blood urea nitrogen. Cr.Cl: Creatinine clearance. *significant correlation

Table (٤): Predictive value for the evaluated predictors of AKI in the studied groups using ROC curve

Predictors	Group B			Group D		
	AUC	Significance	٩٥٪ CI	AUC	Significance	٩٥٪ CI
Preoperative **EF**	٠.٦٢	٠.٠٨	٠.٤٨-٠.٧٧	٠.٥٦	٠.٥٧	٠.٣٥-٠.٧٦
Preoperative **serum Cr**.	٠.٥٣	٠.٦٠	٠.٣٩-٠.٦٨	٠.٦٩	٠.٠٧	٠.٤٧-٠.٩١
Preoperative **BUN**	٠.٥٦	٠.٤٢	٠.٤٠-٠.٧١	٠.٦٤	٠.٢١	٠.٤٠-٠.٨٨
Preoperative **uNGAL**	٠.٥٧	٠.٣٢	٠.٤١-٠.٧٢	٠.٦٢	٠.٢٦	٠.٤٣-٠.٨٠
Postoperative **uNGAL**	٠.٩٧	٠.٠٠٠١*	٠.٩٢-١	٠.٩٥	٠.٠٠٠١*	٠.٨٩-١

AUC: Area under curve. CI: Confidence Interval. EF: Ejection fraction. Cr.: Creatinine. BUN: Blood urea nitrogen. uNGAL: Urinary neutrophil gelatinase-associated lipocalin. *significant predictive value

Table (٥): Sensitivity and specificity of different cut-off values of postoperative urinary neutrophil gelatinase-associated lipocalin (uNGAL) in the studied groups using receiver operating characteristics curve

Cut off value (ng/mL)	Group B		Group D	
	Sensitivity	Specificity	Sensitivity	Specificity
١٩٠	٩٦٪	٩٧٪	٩٦٪	٧٠٪
٢٥٠	٨٦٪	١٠٠٪	٩٣٪	٨٠٪
٤٥٠	٧٠٪	١٠٠٪	٧٣٪	٩٠٪

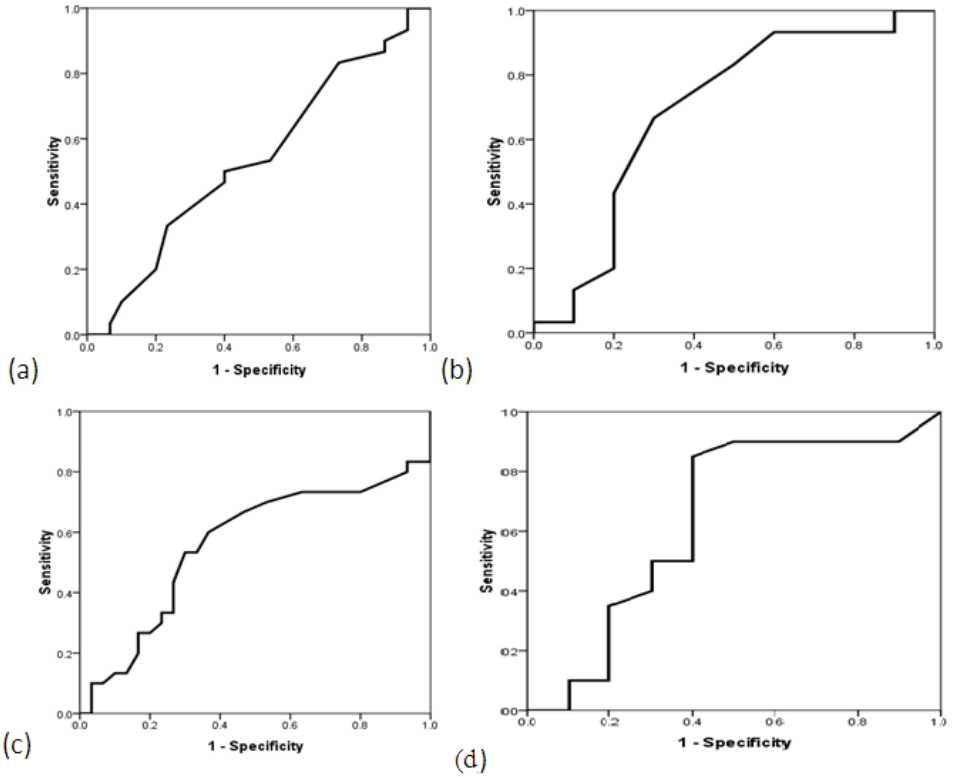

Fig. (١): ROC curves for serum creatinine and blood urea for early detection of AKI after cardiac surgery: a) serum creatinine in group B; b) serum creatinine in group D; c) blood urea in group B; and d) blood urea in group D

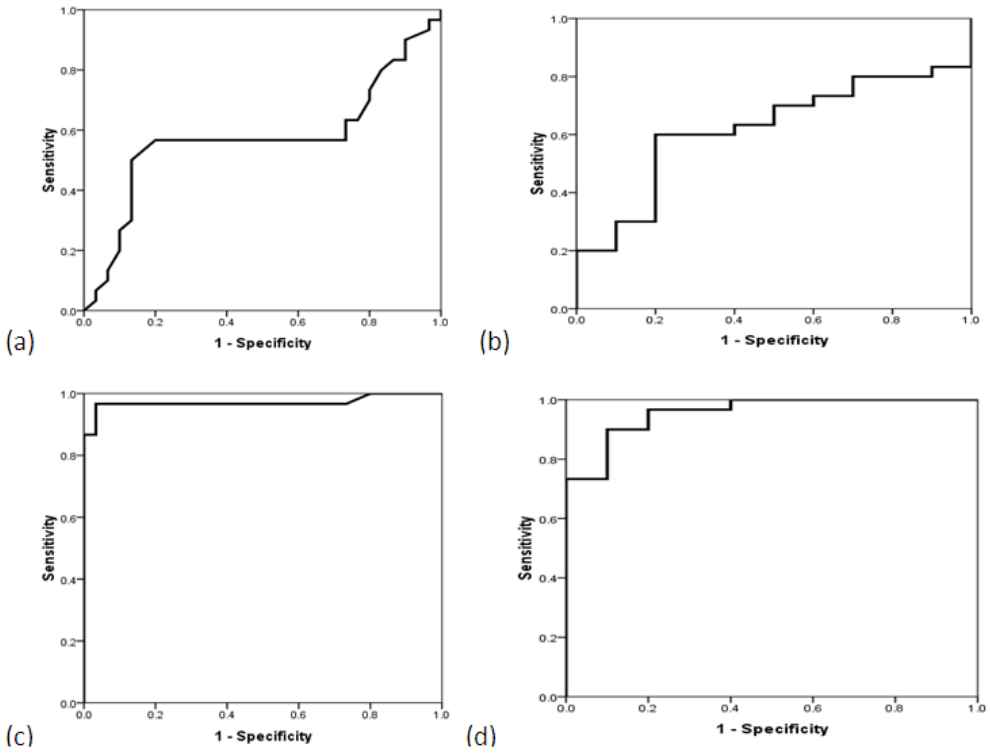

Fig. (٢): ROC curves for preoperative and postoperative urinary NGAL for early detection of AKI after cardiac surgery: a) preoperative urinary NGAL in group B; b) preoperative urinary NGAL in group D; c) postoperative urinary NGAL in group B; and d) postoperative urinary NGAL in group D

Discussion

Both serum and urinary NGAL (uNGAL) have been found to be reliable predictors of AKI in cardiac surgical patients[١, ١٠-١٣]. The study of this novel biomarker in the present study and previous studies focused on the desirable characteristics of clinically applicable AKI biomarkers include: (i) they should be non-invasive and easy to perform at the bedside or in a standard clinical laboratory, using easily accessible samples such as blood or urine; (ii) they should be rapidly and reliably measurable using standardized clinical assay platforms; (iii) they should be sensitive to facilitate early detection, and with a wide dynamic range and cut-off values that allow for risk stratification; and (iv) they should exhibit strong biomarker performance on statistical analysis, including accuracy testing by receiver operating characteristic curves[١٤].

In the present study, the total CPB time and cross-clamp time were significantly higher in patients with AKI than those who did not develop postoperative AKI. The relation of prolonged CPB and cross-clamp times with AKI can be is most likely results from subsequent prolonged transient ischemia reperfusion injury sustained by the kidney [١٢].

In the present study, the postoperative levels of uNGAL were significantly higher in patients with AKI than those who did not develop postoperative AKI. The rapid increase of NGAL in urine after AKI may be explained by a rapid induction of NGAL messenger RNA after brief periods of ischemia within the first few hours peaking at about ٢٤ hours post injury [١٥]. The significant increase of postoperative uNGAL in patients with AKI is supported by results of multiple recent studies in literature which estimated the levels of uNGALin adult patients with AKI after cardiac surgery[١٦-٢٠].

In the present study, using ROC curves the postoperative uNGAL had a significant high predictive value of AKI with AUC of ٠.٩٧ in group B and ٠.٩٥ in group D. The large AUC revealed that postoperative uNGAL can be considered as a useful diagnostic test for AKI after cardiac surgery. These findings are supported by the findings obtained in other recent studies used ROC curves to estimate the diagnostic accuracy of urinary NGAL for diagnosis of AKI after cardiac surgery in adult patients [١٢, ١٨-٢٠].

In the present study, the best cutoff value of uNGAL to determine the AKI after cardiac surgery was ١.٩ ng/dL(١٩٠ ng/mL) in group B which yielded a sensitivity of ٩٦٪ and specificity of ٩٧٪, and it was ٢.٥ ng/dL (٢٥٠ ng/mL) in group D which yielded a sensitivity of ٩٣٪ and specificity of ٨٠٪. In literature, there is no fixed cutoff for uNGAL for diagnosis of AKI using ROC curves.

In the study by Bennett et al., (٢٠٠٨), for a cutoff value of١٠٠ ng/ml, the sensitivity was ٨٢٪, and the specificity was ٩٠٪ for prediction of AKI [١٠]. In the study by Wan et al., (٢٠٠٨) for concentrations in urine of NGAL at ٢ h after surgery, sensitivity was ٧١٪, and specificity was ٧٣٪ for a cutoff value of ٢٥٠ ng/mL [٢١]. In the study by Tuladhar et al., (٢٠٠٩) setting a threshold of ٣٩٣ ng for urinary NGAL per millimole creatinine yielded a sensitivity and specificity of ٩٢.٧٪ and ٧٧.٨٪, respectively [١٢]. The study by Munir et al., (٢٠١٣) analysis of urine NGAL at a cutoff value of ٨٧ng/ml showed sensitivity of ٩٠.٩٪ and specificity of ٩٨.٧٪ [٢٠].

In addition to its diagnostic accuracy determined in the present study and in other studies, the study by Shaw et al., (٢٠١١) concluded that the use of urinary NGAL after cardiac surgery appears to be cost-effective in the early diagnosis of AKI [٢٢].

The strength of the present study may be attributed to: ١) conducting the study in multi-centers; and ٢) reporting the sensitivity, specificity and AUC for the diagnosis of AKI, which are essential to determine the accuracy of the biomarker.

Despite this, there are some limitations to our study: ١) relatively small sample size; ٢) the predicted cutoffs for urine NGAL may be different from that obtained in other populations; ٣) we did not directly calculate glomerular filtration rate, rather using serum creatinine which is chosen because it is currently considered as the clinical gold standard. However, using AKI as defined by a change in serum creatinine sets up the biomarker assay for lack of accuracy due to

either false positives (true tubular injury but no significant change in serum creatinine) or false negatives (absence of true tubular injury, but elevations in serum creatinine due to pre-renal causes or any of a number of confounding variables that haunt this measurement); ٤) the present study did not include patients with chronic kidney disease (CKD). This is problematic, not only because it excludes a large proportion of subjects who frequently develop AKI in clinical practice, but also because CKD in itself can result in increased concentrations of NGAL, thereby representing a confounding variable; and ٥) we estimated the accuracy of urinary NGAL, however simultaneous examination of other urinary biomarkers as potential predictors of AKI may be more informative.

It will be important in future studies to demonstrate: (١) association between biomarkers and preoperative high risk conditions; (٢) association between biomarkers and clinical outcomes such as dialysis, cardiovascular events and death; and (٣) randomization to a treatment for AKI based on high biomarker levels results in an improvement in kidney function and reduction of adverse clinical outcomes.

Conclusion

In conclusion, urinary NGAL is an early predictive biomarker for acute kidney injury AKI occurring in adults after cardiac surgery using CPB. Urinary NGAL when compared with serum creatinine is an earlier predictor of acute kidney injury AKI. Urinary NGAL, due to its high sensitivity, specificity and accuracy, is valuable biomarker for early detection of CSA-AKI.

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الاكتشاف المبكر للخلل الحاد بوظائفالكلى بقياس معامل الليبوكالين المرتبط بجيلاتينيزالنيوتروفيل البولي بعد جراحات القلب المستخدمة لماكينة القلب والرئة الصناعية .

خلفية البحث: الإصابة الحادة بالكلى غالبا ما تحدث بعد جراحات القلب المستخدمة لماكينة القلب والرئة الصناعية و يصل معدل الإصابة إلى ٣٠ ٪ من جميع المرضى . ويؤدي هذا المرض إلى زيادة نسبة المرضية والوفيات ، ومدة العلاج في الرعاية المركزة . هناك حاجة إلى إيجاد مؤشرات حيوية متاحة و سريعة وحساسة و دقيقه تسمح بالتنبؤ المبكر بالإصابة الحادة بالكلى. مؤخرا ، أظهرت العديد من الدراسات أن معامل الليبوكالين المرتبط بجيلاتينيزالنيتروفيل و يرمز لهNGAL يتواجد بشكل كبير في الكلى البشرية الأنابيب القشرية والدم و البول بعد إصابات كلى الحادة وبالتالي معامل NGAL البولي يعتبر من العلامات البيولوجية الدالة على حدوث إصابات كلى الحادة في وقت مبكر .

الهدف من البحث:دراسة جدوى استخدام معامل الليبوكالين المرتبط بجيلاتينيزالنيتروفيل البولي و يرمز له uNGAL للاكتشاف المبكر لإصابات كلى الحادة بعد جراحات القلب المستخدمة لماكينة القلب والرئة الصناعية.

المرضى و طرق البحث: أجريت هذه الدراسة على مرحلتين الأولى في مصر في قسم جراحة القلب بمستشفى جامعة عين شمس في القاهرة و مستشفى جامعة المنيا في المنيا و المرحلة الثانية في ايطاليا في قسم جراحة القلب بمستشفى سانتا كروتشي في مدينو كونيو .وشملت الدراسة ١٠٠ مريضا بالغا خضعوا لعمليات جراحية في القلب باستخدام ماكينة القلب والرئة الصناعية و صنفت حالات الرسالة في أربع مجموعات :المجموعة الأولى : ٣٠ مريضا من مصر لم يحدث لهم إصابة حادة بالكلى بعد الجراحة .المجموعة الثانية : ٣٠ مريضا من مصر حدث لهم إصابة حادة بالكلى بعد الجراحة .المجموعة الثالثة : ١٠ مريضا من إيطاليا لم يحدث لهم إصابة الحادة بالكلى بعد الجراحة .المجموعة الرابعة : ٣٠ مريضا من إيطاليا حدث لهم إصابة حادة بالكلى بعد الجراحة .كما تم تعريف الإصابة الحادة بالكلى بزيادة في نسبة الكرياتينين بالدم في فترة ما بعد الجراحة بأكثر من ٠.٥ ملغ / ديسيلتر (٠.٤٤ مل مول / لتر) عن نسبته قبل الجراحة على النحو المحدد في المؤتمر التوافق للفشل الكلوي الحاد عام ٢٠٠٣ .

نتائج البحث: قياس معامل الليبوكالين المرتبط بجيلاتينيزالنيتروفيل البولي و يرمز له uNGAL مبكرا بعد الجراحة (في غضون ساعتين بعد انتهاء الجراحة) هو اختبار صالح للتنبؤ المبكر بإصابات كلى الحادة بعد جراحات القلب المستخدمة لماكينة القلب والرئة الصناعية ، وذلك لأن هذا المعامل له حساسية و دقة ونوعية عالية.

الاستنتاج: قياس معامل الليبوكالين المرتبط بجيلاتينيزالنيتروفيل البولي و يرمز له uNGAL مبكرا في غضون ساعتين بعد انتهاء الجراحة يمكن قبوله كمعامل للتنبؤ المبكر بإصابات كلى الحادة بعد جراحات القلب المستخدمة لماكينة القلب والرئة الصناعية .