Research Article

The role of neutrophil gelatinase-associated lipocalin (NGAL) in predicting acute kidney injury after cardiac surgery

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Abstract
Cardiac surgery-associated acute kidney injury (CSA-AKI) is a common and serious postoperative complication of cardiac surgery. Acute kidney injury (AKI) is defined as increase in SCr by > ٠.٣ mg/dl (> ٢٦.٥µmol/l) within ٤٨ hours; or increase in SCr to >١.٥ times baseline, which is known or presumed to have occurred within the prior ٧ days; or urine volume < ٠.٥ ml/kg/h for ٦ hours. Since the early stages of AKI are often reversible, AKI should be prevented and/or treated by various approaches instituted as early as possible after the initiating insult, well before serum creatinine even begins to rise. There are promising candidate biomarkers with the ability to detect an early AKI; Neutrophil gelatinase-associated lipocalin (NGAL) is one of such biomarkers. **The aim of this work** is to assess the role of serum NGAL as early biomarker for prediction of acute kidney injury, before the rise in serum creatinine, following cardiac surgery.
The study was conducted on ٢٥ patients selected from patients who were subjected to cardiac surgery at Cardio-Thoracic Surgical Department of Minia University Hospital and proved that they developed A K I after the surgery. The study also included ١٥ apparently healthy volunteers who served as a control group of matched age and sex. Serum NGAL by ELISA was measured to patients group (group I) on ١st day post operative (٦ hours after discharge from surgical room), ٢nd day and ٣rd day with ٢٤ hours interval and applied also to subjects of control group (group II). The ROC analysis has been extensively used as a fundamental evaluation tool. It showed that serum NGAL of patients group on **١st day and ٢nd day** post operative was a **good marker** for prediction of AKI, Concerning ٣rd **day** post operative it showed that NGAL was an **excellent marker** for prediction of AKI. Conclusions: Serum NGAL was early biomarkers of AKI prior to rise in serum creatinine and has high positive predictive values for occurrence of AKI following cardiac surgery.

Key Words:
kidney injury**,** Neutrophil gelatinase, lipocalin, Cardiac surgery

Introduction
Acute Kidney injury (AKI) is one of a number of conditions that affect kidney structure and function. It is defined by an abrupt decrease in kidney function that includes, but is not limited to, acute renal failure (ARF) (Hoste et al., ٢٠٠٦ and Uchino et al., ٢٠٠٦).

AKI is defined as any of the following (Not Graded): Increase in serum creatinine by > ٠.٣ mg/dl (>٢٦.٥ µmol/l) within ٤٨ hours; or Increase in SCr to >١.٥ times baseline, which is known or presumed to have occurred within the prior ٧ days; or urine volume <٠.٥ ml/kg/h for ٦ hours (Bellomo et al., ٢٠٠٤ and Mehta et al., ٢٠٠٧).

To create uniformity in the diagnosis of AKI, the RIFLE (risk of renal injury/injury to the kidney/failure of kidney function/loss of kidney function/end-stage disease) criteria were proposed in ٢٠٠٤ (Bellomo et al., ٢٠٠٤), followed by the Acute KidneyInjury Network (AKIN) criteria in ٢٠٠٧ (Mehta et al., ٢٠٠٧) and the Kidney Disease–Improving Global Outcomes (KDIGO) criteria in ٢٠١٢ Kidney International KDIGO ٢٠١٢. These

criteria express the deterioration of kidney function as a decline in the estimated glomerular
filtration rate (eGFR), which is based on an increasing serum creatinine (SCr) concentration and a declining urine output (de Gues et al., ٢٠١٦).

AKI is a broad clinical syndrome encompassing various etiologies, including specific kidney diseases (e.g., acute interstitial nephritis, acute glomerular and vasculitic renal diseases); non-specific conditions (e.g, ischemia, toxic injury); as well as extrarenal pathology (e.g., prerenal azotemia, and acute postrenal obstructive nephropathy) (Bellomo et al., ٢٠٠٨ and Gaffney et al., ٢٠١٥).

Worldwide, more than ٢ million cardiac surgeries are performed each year. Cardiac surgery–associated acute kidney injury (CSA-AKI) is a serious postoperative complication, and is the second most common cause of AKI in the intensive care unit (Uchino et al., ٢٠٠٥).

An incidence of CSA-AKI of up to ٣٩٪ has been reported, varying depending on patient-related baseline characteristics and the type of surgery (Mao et al., ٢٠١٣). Between ٣٪ and ٦.٥٪ of all surgical patients require renal replacement therapy. This worst stage of CSA-AKI is independently associated with a very high mortality rate (Wald et al., ٢٠١٥).

Other clinical consequences of CSA-AKI are increased length of hospital stay, increased risk of chronic kidney disease (CKD), and increased risk of death within ٥ years after surgery (Hansen et al., ٢٠١٣).

The success of interventions or new therapeutic strategies aimed at reducing the incidence of CSA-AKI and its related outcomes depends on the optimum time of their application, which is at the very early stages of AKI (de Gues et al., ٢٠١٦).

However, in health, the kidneys have a significant degree of excess capacity, such that ٥٠٪ of the functional kidney mass can be damaged without any drop in SCr-based eGFR. Therefore, an increase in SCr occurs relatively late after the initial injurious event (٢٤-٤٨ hours), with hemodilution related to pump priming as an additional contributor to this delay (Perrone et al., ١٩٩٢ and Coca et al., ٢٠٠٨). The field of AKI biomarkers is rapidly evolving, and new proteins released by injured tubular cells are constantly being discovered. All of these new biomarkers carry the potential to serve as markers for acute tubular damage in the absence of functional AKI (Devarajan et al., ٢٠٠٨). Most promising candidate is neutrophil gelatinase-associated lipocalin (NGAL).

NGAL: Neutrophil gelatinase−associated lipocalin (NGAL) is ٢٥ kDa iron−transporting glycoprotein which accumulates in the kidney tubules and urine after nephrotoxic and ischemic insults (Mishra et al, ٢٠٠٣). Human NGAL was originally isolated from the supernatant of activated neutrophils. It has been proposed as an early, sensitive, noninvasive biomarker for AKI (Sargentini et al., ٢٠١٢).

NGAL is a small siderophoric protein that is intensely up-regulated and excreted in cases of acute tubular damage. It can be detected in both plasma and urine (Mishra et al., ٢٠٠٤).

In the early phases of AKI, NGAL mitigates iron-mediated toxicity by providing a reservoir for excess iron, and in subsequent phases, it promotes regeneration and repair by regulating intracellular iron availability (Mishra et al., ٢٠٠٦).

NGAL is readily filtered in the glomerulus and readily reabsorbed in the proximal tubular segments (Mori et al., ٢٠٠٥). Immediately following diverse injurious events, NGAL is up-regulated in the distal parts of the nephron (Devarajan ٢٠٠٧).

Subjects and Methods

The study was conducted on ٢٥ patients selected from patients who were subjected to cardiac surgery at Cardio-Thoracic Surgical

Department of Minia University Hospital, through the period from September,٢٠١٤ to June, ٢٠١٥ and proved that they developed A K I after the surgery as manifested by an absolute increase in serum creatinine of ٠.٣mg/dl or more , equivalent to a percentage increase in serum creatinine ٥٠٪ or more (١.٥-fold from base line) within ٤٨ hours according to RIFLE and AKIN criteria The study also included ١٥ apparently healthy volunteers who served as a control group of matched age and sex .

Excluded from the study were patients on nephrotoxic drugs Exclusion criteria those with pre-existing renal insufficiency, Patients on immunosuppressive therapy and /or patients with active infection at time of study.

All the study individuals were subjected to-Thorough clinical history, Clinical examination, routine Laboratory investigations and special Laboratory investigations: Serum NGAL by ELISA which applied to patients of group I on ١st day post operative (٦ hours after discharge from surgical room), ٢nd day and ٣rd day with ٢٤ hours interval and applied also to subjects of control group (group II).

Statistical analysis

All analyses were performed with version ١٩ of Statistical Package of Social Science (SPSS). Qualitative data were expressed as proportions, while quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were analyzed by Chi square ($\lambda^{٢}$) test. Comparisons between groups for normally distributed quantitative data were performed by Student's t-test. Correlations between variables were obtained by Pearson's test.

For all analysis, statistical significance was defined as p value less than ٠.٠٥.
The ROC analysis has been extensively used as a fundamental evaluation tool in clinical studies pertaining to diagnostic testing. A ROC curve is a graphical plot of the sensitivity on the y-axis versus (١-specificity) on the x-axis for a binary classifier system as its discrimination threshold is varied. For biomarker analysis, the binary classification task is typically to determine whether a subject has a certain disease (such as AKI) or not. Characteristically, ROC curves are generated for various cut-off points for the biomarker concentration under consideration. A commonly derived statistic from the ROC curve is the area under the curve (AUC). An AUC of ١.٠ represents a perfect biomarker, whereas an AUC of ٠.٥ indicates a result that is no better than expected by random chance. An AUC of ٠.٧٥ or above is generally considered a good biomarker, and an AUC of ٠.٩ or above would represent an excellent biomarker (Zou et al., ٢٠٠٧).

Results

The study showed that there was significant increase of serum NGAL in patients group on ١st day, ٢nd day and ٣rd day following the cardiac surgery when compared with control group as shown in table (١).

Table (١): Results of serum NGAL

Variable	Group I (n=٢٥)	Group II (n=١٥)	P - value
NGAL ١st day µg/dl			
Range	٠.٠٣ – ٣.٦	٠.١ -٠.٩٠	
Mean±SD	١.٣٩±١.١٤	٠.٣١±٠.٢٦	P=٠.٠٠٠٣
NGAL ٢nd day			
Range	٠.٠٦ – ٦.٥	٠.١ – ٠.٩٠	P=٠.٠٠٢
Mean±SD	١.٦٨±١.٧٦	٠.٣١±٠.٢٦	
NGAL ٣rd day			
Range	٠.٢ –٤.٢	٠.١ – ٠.٩٠	P= ٠.٠٠٠
Mean±SD	١.٧٠±١.١٦	٠.٣١±٠.٢٦	

As demonstrated in figures ١, ٢ and ٣, Roc curves showed an AUC of ٠.٨٤ (p<٠.٠٠٠١), an AUC of ٠.٨٠ (p<٠.٠٠٢) and an AUC of ٠.٩٠ (p<٠.٠٠٠١) for ١st day ,٢nd day and ٣rd day post operative respectively.

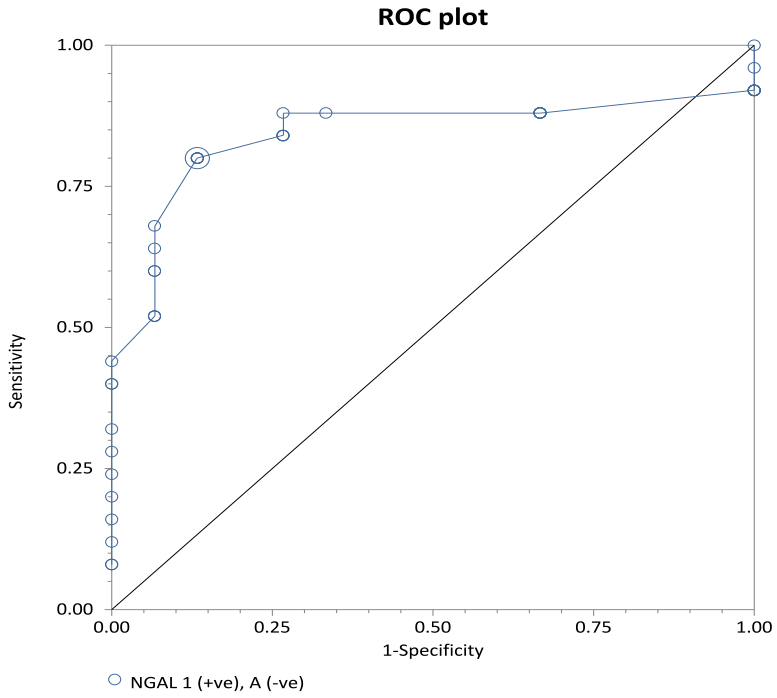

Figure (١): ROCcurve of NGAL on ١st day

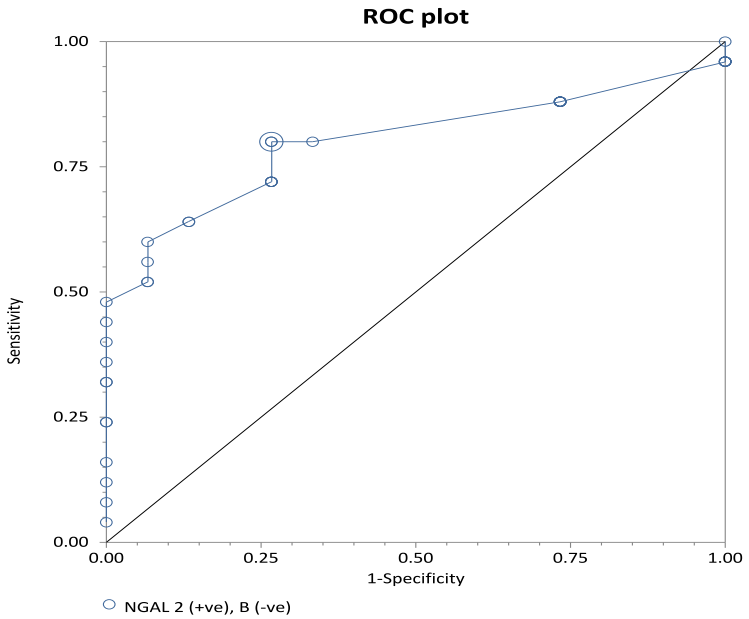

Figure (٢): ROCcurve of NGAL on ٢nd day

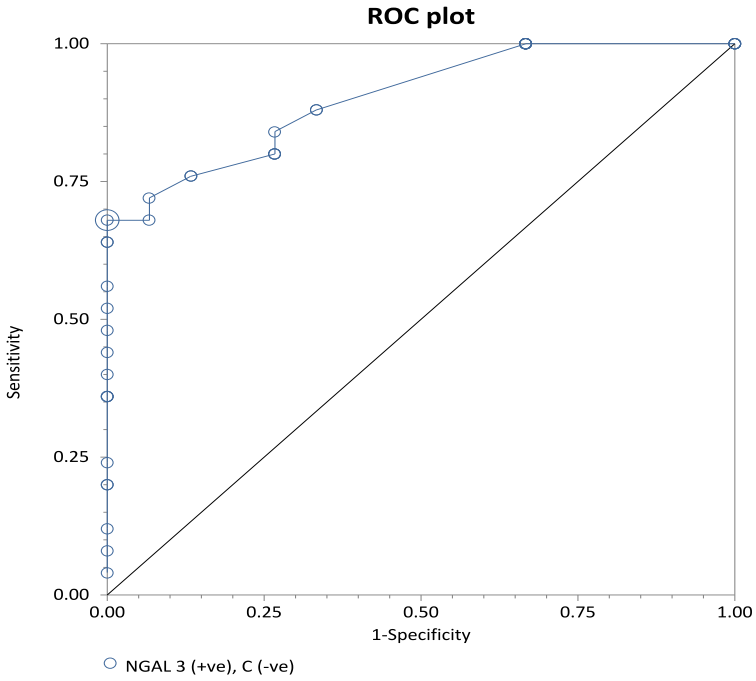

Figure (٣): ROCcurve of NGAL on ٣rd day

In the study ,**serum NGAL** showed significant positive correlation with creatinine on ١st day post operative(r= ٠.٥٦; p= ٠.٠٠٤), ٢nd day (r= ٠.٦٤ ; p= ٠.٠٠٠٦) and ٣rd day (r= ٠.٥٥ ; p=٠.٠٠٤) .

Discussion

In this study serum NGAL had been found that there was significant increase in serum NGAL in patients group on ١st day, ٢nd day and ٣rd day post operative than control group. Using ROC curve analysis, Our study showed that serum NGAL considered a good marker for prediction of AKI at ١st day (٦ hours post operative) and ٢nd day. An excellent marker at ٣rd day following cardiac surgery

These findings were in agreement with other studies; Jain et al., (٢٠١٦) were able to verify the role of urinary NGAL as an early biomarker of AKI in off pump coronary bypass surgery.

Perhaps the strongest evidence supporting the role of NGAL as an early biomarker for AKI in cardiac surgery patients, came from the meta analysis conducted by Zhou et al., (٢٠١٦) Neutrophil gelatinase-associated lipocalin (NGAL) has been identified as one of the most sensitive and specific biomarkers for predicting cardiac surgery-associated acute kidney injury (CSA-AKI).

Moreover in another study conducted by deGues et al., (٢٠١٦) they also were able to document the role of NGAL in early diagnosis of CSA-AKI.

Moreover Bennett et al., (٢٠٠٨) in a prospective uncontrolled cohort study concluded that NGAL predicts AKI, mortality and morbidity after pediatric cardiac surgery. Several other studies have reported NGAL as a sensitive and specific predictor of AKI after cardiac surgery (Krawczeski et al., ٢٠١١ and Mishra et al., ٢٠٠٥).

Conclusions

According to the study, serum NGAL is an early biomarker of AKI prior to rise in serum creatinine, and showed a high positive predictive values for occurrence of AKI following cardiac surgery. Accordingly it can be used for early detection of AKI.

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