The role of neutrophil gelatinase-associated lipocalin in predicting acute kidney injury in patients undergoing off-pump coronary artery bypass graft: A pilot study

Vishal Jain, Yatin Mehta, Abhinav Gupta, Reetesh Sharma, Arun Raizada, Naresh Trehan

Department of Critical Care and Anaesthesiology, Medanta Institute of Critical Care and Anaesthesiology, Medanta Institute of Kidney and Urology, Department of Biochemistry, Chairman and Managing Director, CTVS, Medanta - The Medicity, Gurgaon, Haryana, Department of Critical Care and Emergency, Sharda University, Greater Noida, Uttar Pradesh, India

ABSTRACT

Objective: Acute kidney injury (AKI) is a commonly encountered postoperative complication after cardiac surgery especially in high risk patients. AKI though seen more commonly after conventional on pump coronary artery bypass surgery (CCABG), is not uncommon after off pump coronary bypass surgery (OPCAB). Various biomarkers have shown promise over last one decade as an early marker for predicting AKI postoperatively. NGAL is one such biomarker whose concentration is increased in urine after any nephrotoxic and ischemic insult. The objective of this study was to assess the role of urine NGAL in predicting AKI after OPCAB in patients with increased risk of developing AKI. Design: A prospective cohort study. Setting: A clinical study in a multi-specialty hospital. Participants: Eighty patients. Materials and Methods: study was approved by the hospital research ethics committee. 80 patients posted for OPCAB with an increased risk of developing AKI defined as having a Cleveland Clinic Foundation Acute renal failure scoring System score of ≥6 were included in the study. Patients with coronary angiography (CAG) within 48 hrs prior to surgery, pre-existing AKI, preoperative renal replacement therapy (RRT) and CKD stage 5 were excluded. Urine NGAL level before the start of surgery baseline and at 4 hrs post surgery were done. Renal function tests were assessed on the day of surgery (4 hrs post surgery) and on the next three days. Result: Seven patients developed AKI as defined by acute kidney infection network (AKIN) and risk injury failure loss end stage (RIFLE) criteria for AKI. NGAL value at 4 hrs in patients who developed AKI was significantly higher than in those patients who did not develop AKI (P < 0.05). Conclusion: urine NGAL is an early biomarker of acute kidney injury in patients undergoing OPCAB surgeries. However, large multicentre studies may be needed to confirm it.

Key words: Acute kidney injury; Neutrophil gelatinase associated lipocalin; Off pump coronary artery bypass

INTRODUCTION

Acute kidney injury (AKI) is not uncommon after cardiac surgery and is associated with increased morbidity and mortality. Up to one-third of the patients undergoing cardiac surgery can develop some form of AKI, and 1–5% of these patients may go on to develop a severe form of kidney injury, requiring dialysis.1-3

There is increasing evidence that AKI is associated with increased risk of both...
short-term and long-term risk of death, as well as the risk of progressive renal failure. Even a subtle increase in serum creatinine levels resulting from a mild AKI can increase the risk of 30 days mortality to 3-fold, and larger serum creatinine increases are associated with a more than 18-fold elevation.\[^4,5\]

There are a number of preoperative risk factors which are associated with increased likelihood of developing AKI after cardiac surgery. Prominent among those include female gender, reduced left ventricular function, history of congestive heart failure, diabetes requiring insulin, preoperative use of an intra-aortic balloon pump (IABP), chronic obstructive pulmonary disease (COPD), the need for emergent surgery, and an elevated preoperative serum creatinine. Valve replacement surgeries have higher incidence of AKI as compared to coronary artery bypass graft (CABG) surgery.\[^6-10\]

Another controversial risk factor is cardiopulmonary bypass, i.e., conventional CABG (CCABG) versus off-pump CABG (OPCAB). Most of the studies have shown that OPCAB is associated with lower incidence of AKI postoperatively as compared to CCABG. Patients undergoing OPCAB have a significantly lesser increase in inflammatory markers as compared to those who undergo CCABG. Nigwekar \textit{et al.} reported a significant reduction in overall AKI (odds ratio [OR] 0.57, 95% confidence interval [CI] 0.43–0.76) and AKI-requiring renal replacement therapy (RRT) (OR 0.55, 95% CI 0.43–0.71) in OPCAB cases compared from CCABG.\[^11,12\] A meta-analysis of 22 randomized controlled trials suggested that OPCAB performed in a relatively heterogeneous patient population with coronary artery disease was associated with a reduction in the incidence of AKI but had no effect on dialysis requirement or all-cause mortality.\[^13\]

Serum creatinine is a degradation product of muscle cells and represents a surrogate for the efficiency of glomerular filtration. However, it has a poor predictive accuracy for kidney injury, particularly in the early stages of AKI.\[^14\]

Neutrophil gelatinase-associated lipocalin (NGAL) is 25 kDa iron-transporting glycoprotein which accumulates in the kidney tubules and urine after nephrotoxic and ischemic insults. Human NGAL was originally isolated from the supernatant of activated neutrophils. It has been proposed as an early, sensitive, noninvasive biomarker for AKI.\[^15-21\] We conducted a pilot study to assess the role of NGAL in predicting AKI in patients undergoing OPCAB, who were at increased risk of developing AKI.

**MATERIALS AND METHODS**

This was a prospective cohort study, conducted at our tertiary care hospital after taking permission from the institutional review board. Eighty patients posted for OPCAB with an increased risk of developing AKI, defined as having a Cleveland Clinic Foundation acute renal failure scoring system score of ≥6 were included in the study. Venous blood samples were collected on the day of admission for routine investigations including renal function tests. A baseline urine NGAL level before the start of surgery was assessed and another urine NGAL level was assessed at 4 h postsurgery. Renal function tests were assessed on the day of surgery (4 h postsurgery) and on the next 3 days.

All the patients underwent OPCAB using a standard technique as per the institution protocol. All patients were premedicated with lorazepam 2 mg and pantoprazole 40 mg orally a night before and on the morning of surgery. Anesthesia was induced with thiopentone sodium 2–4 mg/kg, fentanyl 4–5 µg/kg and midazolam 0.03–0.04 mg/kg intravenously. Neuromuscular Blockade was achieved with vecuronium bromide (0.1 mg/kg) and following tracheal intubation; anesthesia was maintained with isoflurane and intermittent doses of fentanyl, midazolam, and vecuronium. A median sternotomy was performed and heparinization was done to keep activated clotting time >300 s. Heparin sulfate was reversed with protamine after the completion of revascularization. Patients were shifted to Intensive Care Unit (ICU) immediately after the surgery and were managed as per protocol.

**Inclusion criteria**
- Age group: 18–85 years
- Patients with increased risk of AKI postoperatively, defined as having a Cleveland Clinic Foundation acute renal failure scoring system score of ≥6
- Patients with severe left ventricular dysfunction (left ventricular ejection fraction [LVEF] ≤30%)
- Patients giving valid written informed consent
- Patients planned for elective OPCAB.

**Exclusion criteria**
- Preoperative hematocrit <25%
- Leukocyte-rich blood transfusion <30 days before surgery
- Coronary angiography within 48 h before surgery
- Preexisting AKI
• Preoperative RRT
• Chronic kidney disease stage 5
• Postrenal transplant.

Patients scheduled for OPCAB were examined a day before the procedure, and all the baseline investigations were noted. Demographic data (age, sex, height, and weight), preoperative risk factors and perioperative surgical and anesthetic management were recorded for all the patients. Urine sample for baseline NGAL levels was taken before the start of surgery. Patients were shifted to ICU after surgery, and another urine sample for NGAL was taken 4 h postsurgery. Renal function tests were assessed on the day of surgery at 4 h postsurgery and for the next 3 days.

Diagnostic criteria for defining AKI was taken from Acute kidney injury network (AKIN/RIFLE) criteria, i.e., an AKI with a Rapid time course (<48 h), reduction of kidney function with an absolute increase in serum creatinine of ≥0.3 mg/dl (≥26.4 µmol/l) or a percentage increase in serum creatinine of ≥50% and a reduction in urine output, defined as <0.5 ml/kg/h for more than 6 h.

Statistical methods
All the values are expressed as terms of means and standard deviation. Qualitative/categorical data are expressed in terms of absolute number and percentages. All statistical tests were two-tailed. Mann–Whitney U-test was used for comparison and a $P < 0.05$ was considered statistically significant. Statistical analysis was performed in SPSS version 18.0 (IBM, SPSS Statistics, Bangalore, India).

RESULTS

Eighty patients who were planned to undergo OPCAB at our center were enrolled in this prospective study. Baseline demographics are shown in Table 1. Mean age of the patients in our study was 64.18 ± 8.88 years with 90% male patients and 10% of patients being female. All the patients had a preoperative LVEF of ≤30%. All the patients had IABP inserted at least 1 day before the surgery. In addition to that, all the patients in the study were diabetics. Five patients had a history of COPD and four surgeries in the study were redo-surgeries.

Seven patients (8.75%) developed AKI as defined by AKIN and RIFLE criteria for AKI [Table 2]. Six patients who developed AKI had stage one AKI while one patient had stage 2 AKI. One patient had a very high value of NGAL both preoperatively and 4 h after the surgery, suggesting that the patient already had developed AKI before the surgery and was therefore excluded from the study. Mean baseline NGAL value in patients who developed AKI, although higher but was comparable with those who did not develop AKI (20.05 ng/ml vs. 15.14 ng/ml, respectively) [Table 3 and Figure 1]. NGAL value at 4 h in patients who developed AKI was 385.58 ng/ml, which was significantly higher as compared to patients who did not develop AKI (35.42 ng/ml, $P < 0.05$). Serum creatinine levels at baseline and at 4 h postoperatively were comparable in patients who develop AKI and those who did not develop AKI ($P > 0.05$). However, serum creatinine levels at 24, 48, and 72 h were significantly higher in patients who developed AKI as compared to those who

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**Table 1: Patient demographics**

| Study Parameter       | Value          |
|-----------------------|----------------|
| Age in years          | 64.18±8.88     |
| Male, n (%)           | 72 (90.0)      |
| DM, n (%)             | 80 (100.0)     |
| HTN, n (%)            | 64 (80.0)      |
| IABP, n (%)           | 80 (100.0)     |
| COPD, n (%)           | 5 (6.25)       |
| Previous cardiac surgery, n (%) | 4 (5.0)        |

**Table 2: Distribution of acute kidney injury**

| AKI | n (%) |
|-----|-------|
| Yes | 7 (8.75) |
| No  | 73 (91.25) |
| Total | 80 (100.0) |

**Table 3: Comparison of neutrophil gelatinase-associated lipocalin and serum creatinine at various time intervals in acute kidney injury and no acute kidney injury patients**

| Parameters     | AKI (n=7) | No AKI (n=73) | P     |
|----------------|-----------|---------------|-------|
| NGAL (ng/ml)   |           |               |       |
| Base line      | 20.05±11.52 | 15.14±11.73   | 0.740 |
| At 4 h         | 385.58±32.54 | 35.42±32.54  | 0.000*|
| Serum creatinine (mg %) |           |               |       |
| Preoperative   | 1.23±0.14  | 1.07±0.18     | 0.062 |
| At 4 h         | 1.21±0.25  | 1.07±0.19     | 0.074 |
| At 24 h        | 1.71±0.50  | 1.03±0.15     | <0.0001*|
| At 48 h        | 1.89±0.32  | 1.03±0.14     | <0.0001*|
| At 72 h        | 2.13±0.42  | 1.05±0.14     | <0.0001*|

Mann–Whitney U-test, *$P<0.05$, statistically significant.
NGAL: Neutrophil gelatinase-associated lipocalin, SD: Standard deviation, AKI: Acute kidney injury
did not develop AKI [Table 3 and Figure 2]. None of
the patients who developed AKI required dialysis. There
was no mortality in the 7 days postoperative period.

DISCUSSION

Serum creatinine has long been used as a marker of renal
function in spite of it being an unreliable and delayed
indicator of renal injury. Serum creatinine in a setting
of AKI may take several hours to days to reach a steady
state. Furthermore, serum creatinine level is influenced by
several nonrenal factors, such as age, gender, muscle mass,
muscle metabolism, medications, and hydration status.

Availability of early biomarkers in predicting AKI in
cardiac surgery may enable the physicians to perform
timely interventions to prevent further deterioration of
kidney functions. A number of biomarkers have
recently been investigated as possible biomarkers for
early and accurate identification of AKI. These include
plasma biomarkers such as NGAL and cystatin C and
urine biomarkers such as NGAL, interleukin-18, and
kidney injury molecule-1.[22-24]

NGAL is one such novel biomarker, which is primarily
a marker of renal tubular injury There is a marked
up-regulation of NGAL mRNA in the thick ascending
limb of Henle’s and the collecting ducts,[25] with
resultant synthesis of NGAL protein in the distal
nephron and secretion into the urine where it comprises
the major fraction of urinary NGAL.

We found a significant relationship between a rise
in urine NGAL levels at 4 h postoperatively to the
occurrence of AKI, although it was not clear whether
the degree of rise in NGAL levels was associated with
more severe form of AKI or not. This was because of the
reason that six out of seven patients in our study who
developed AKI had only stage one AKI. Furthermore,
the rise in urine NGAL levels at 4 h in these patients
was not uniform. One patient who developed AKI had
100-fold rise in urine NGAL levels at 4 h while the other
six had a lesser degree of elevation of urine NGAL at 4 h.
Six patients who developed AKI had a normal serum
creatinine value at 4 h postoperatively. Serum creatinine
levels rose only at 24 h or more in all these six patients.
However, urine NGAL levels at 4 h were significantly
high in the patients who developed AKI suggesting that
urine NGAL rises much earlier than serum creatinine
levels and is an early biomarker of AKI. One patient who
developed AKI had high urine NGAL levels at 4 h as well
as raised serum creatinine levels at 4 h postoperatively.

Since none of the patient in our study who developed
AKI had grade 3 AKI and neither of the patient had
dialysis requirement, the diagnostic utility of NGAL
may be better assessed by determining its predictive
utility for clinical outcomes such as mortality or
progression to dialysis-dependent renal failure.

One of the strengths of our study was that all the
patients who were selected in the study had a high risk
for developing AKI postoperatively. Various studies
and scoring systems for predicting AKI in patients
undergoing cardiac surgery have shown that patients
with poor left ventricular function and diabetes mellitus are at increased risk of developing AKI after
cardiac surgery. The effect of diabetes mellitus on
postoperative renal dysfunction may be the result of
renal parenchymal diseases, such as glomerulonephritis
or glomerulosclerosis. Renal artery stenosis in diabetic
patients may further compromise renal function.
In addition, perioperative IABP is an independent predictor of perioperative AKI and in-hospital mortality. Wijeysundera et al. also demonstrated that the preoperative placement of an IABP significantly increased the risk for AKI in a heterogeneous group of cardiac surgical patients.[26]

Our study is in agreement with other similar studies which have found that NGAL is an early biomarker of AKI. Bennett et al. in a prospective uncontrolled cohort study concluded that NGAL predicts AKI, mortality, and morbidity after pediatric cardiac surgery.[27] Several other studies have reported NGAL as a sensitive and specific predictor of AKI after cardiac surgery.[28,29]

It also supports the fact that OPCAB may have a protective effect as far as postoperative renal dysfunction is concerned as compared to CCABG, as only 8.75% of patients had AKI in our study which is consistent with the findings in other studies which showed that risk of AKI was much more in CCABG.[30] Loef et al. found significantly less changes in microalbuminuria, free hemoglobin, fractional excretion of sodium, and free water clearance as well as N-acetyl-D-glucosaminidase as a marker for tubular function and damage, respectively, in patients undergoing OPCAB as compared with CCABG patients. Patients with concomitant diseases or risk factors seem to benefit from the beating heart approach as compared to CCABG.[31,32]

**Limitations**

One of the important limitations of our study is that only a small study group (n = 80) was included in the study. Large randomized prospective trials are needed to validate the diagnostic utility of urine NGAL in predicting AKI.

Another limitation of the study is that the urine NGAL level was assessed at 4 h postoperatively and not after that, which is a relatively short period of observation. However, earlier studies have indicated that AKI and a rise in urine NGAL levels occur mostly within this period.

Although all the subjects in the study had normal serum creatinine measurements before surgery, an estimation of glomerular filtration rate to document normal kidney function was not made.

Role of perioperative diuretics in influencing the urine NGAL levels was not considered in the study. Patients without AKI, who received diuretics, may have a marked diuresis thereby diluting urinary NGAL, whereas patients with evolving AKI may have a blunted response to diuretics with less urinary dilution, thus enhancing the discriminant ability of urinary NGAL for AKI.

**CONCLUSION**

In our study, we found a definite relationship between a rising urine NGAL level and AKI after OPCAB. Further larger studies are required to confirm the potential role and limitations of NGAL as an AKI biomarker.

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**Conflicts of interest**
There are no conflicts of interest.

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