Plasma neutrophil gelatinase-associated lipocalin role as a developing biomarker of acute renal failure following coronary artery bypass surgery

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Implication for health policy/practice/research/medical education:
Acute kidney injury afterward CABG procure is related with elevated morbidity and mortality, early diagnosis of which is important for effective management of these patients. Neutrophil gelatinase-associated lipocalin (NGAL) is a secreted glycoprotein in response to inflammation.

Introduction
Coronary heart disease is a key reason of death and disability, especially in developed countries. Coronary artery bypass graft (CABG) operations are one of the most prevalent performed major operations in the world (1). Acute kidney injury (AKI) is a devastating complication after cardiac surgery causing increased morbidity and mortality. Up to 50% of the patients undergoing cardiac surgery develop AKI, and about 5% of these patients lead to renal failure requiring dialysis, which can increase risk of death from 15% to 80% (2,3).

The serum creatinine concentration that usually reflects the balance between the synthesis and the urinary excretion of creatinine is routinely conducted to predict postoperative AKI (4). However, limitations for creatinine as a marker of renal injury in acute conditions are widely appreciated. For example; creatinine is produced in skeletal muscle cells and can provide a surrogate for the efficiency of glomerular filtration. In addition, data suggest that serum creatinine appears to be less indicative of tubular damage. Hence, serum creatinine and urinary output are unreliable for early diagnosis of AKI (3,4).

Neutrophil gelatinase-associated lipocalin (NGAL) is known as a gene that belongs to the lipocalin superfamily and is expressed by neutrophils and other epithelial cells. It is rarely detected in plasma of healthy kidneys; however it is firstly indicated in activated neutrophils and renal tubular cells in reaction to ischemic injury or inflammation (5). According to the previous researches, NGAL can raise in reaction to renal tubular injury in humans and rodents and it seems so rapidly in the urine and serum that it is a valuable kidney biomarker for the early diagnosis of acute renal failure after kidney transplantation (6), renal damage in brucellosis patients receiving gentamycin (7), hemolytic uremic syndrome (8) and lupus nephritis (9), and also AKI after the administration of contrast agents (10). NGAL could be identified in the initial urine samples about two hours after ischemia, therefore it may be of value as a predictor of AKI in acute clinical settings (11).

Although the role of NGAL as a prognostic biomarker for AKI has been approved in some previous studies, there is still controversy in this regard. The results of NGAL gene analysis show relatively high genetic diversity in different populations which may have important clinical consequences. We therefore conducted a cross-sectional, population-based study to determine whether serum NGAL levels predict AKI progression in Iranian patients undergoing CABG. Data indicate that the plasma NGAL levels can be potentially used for early diagnosis of AKI in patients undergoing CABG.

Objectives
The present study was designed to determine whether serum NGAL levels predict AKI progression in patients undergoing CABG.

Patients and Methods
Study design
This work was a cross-sectional prospective study conducted on 79 patients undergoing CABG surgery recruited at Musavi hospital of Zanjan university of medical sciences, Zanjan, Iran from December 2016 to May 2017. Patients who had pre-existing history of kidney or hepatic failure, pulmonary artery hypertension, severe proteinuria (>3.5 g/d), acute or chronic pulmonary disease, previous cardiac surgery, malignancy, infectious illnesses or inflammatory states were excluded from the study. Additionally, patients under therapy with steroids or nephrotoxic drugs (e.g.; aminoglycoside) during the preoperative days were excluded from the analysis too. All participants signed a written informed consent before the study.

The variables of demographic data (age, gender), diabetes, history of myocardial infarction, prior cardiac surgery, vascular or respiratory disease, smoking and renal dysfunction, the form and intensity of coronary artery stenosis were recorded by the questionnaires. Moreover; duration of operation and anesthesia, data regarding hemodynamic parameters, urine output and total amount of drainage were recorded for all patients.

Peripheral blood samples were drawn and collected in sterile heparinized tubes to evaluate the serum creatinine and plasma NGAL levels at baseline (before anesthesia induction), and at 12, 24, and 48 hours post-operation. The plasma NGAL level was determined by enzyme-linked immunosorbent assay (ELISA) method using Crystal Day Biotech Kit (Shanghai, China) according to the manufacture’s instruction. The criteria for the diagnosis of AKI were defined as any of the following issues; the 25% (or higher) increase in the serum creatinine level compared to its baseline value, a rise in serum creatinine level ≥0.3 mg/dL within 48 hours; or urine volume <0.5 mL/kg/h for six hours after operation.

Statistical analysis
The composed data were investigated through SPSS 22 software by descriptive analytical test (Mean ± SD), repeated measures ANOVA, frequency and percentage for presenting descriptive data was conducted. To compare the qualitative data, independent t test was used. Meanwhile, Kolmogorov-Smirnov test was conducted for normality of variables. P value less than 0.05 is known significant.

Results
The study enrolled the number of patients that was similar to the previous studies reported in this area. After coronary artery bypass graft, the patients were separated into two groups based on the occurrence of acute renal failure (Table 1). AKI as a postoperative complication occurred in 23 (29%) of cases, around 53.6% of which were men (Table 1). There were no remarkable difference between
two groups in demographic characteristics, history of diabetic mellitus and history of angiotensin-converting enzyme, angiotensin receptor blockers or non-steroidal anti-inflammatory drugs receiving, while among the study group 87% had a history of hypertension ($P=0.04$; Table 1).

The AKI was not diagnosed by the serum creatinine criteria during the first 24 hours post-operatively (Table 2). The mean serum creatinine levels 12 hours post-operation were $1.23 \pm 0.49$ mg/dL and $1.11 \pm 0.27$ mg/dL in the AKI and non-AKI groups, respectively ($P=0.16$). The mean serum creatinine concentrations at 24 hours postoperative were $1.56 \pm 0.53$ mg/dL and $1.09 \pm 0.30$ mg/dL in the AKI and non-AKI groups, respectively ($P<0.001$; Table 2).

The mean plasma NGAL stage at baseline ($74.9 \pm 33.5$ ng/mL) was significantly lower than after CABG ($144.07 \pm 136.3$ ng/mL) ($P<0.001$). The mean plasma NGAL concentration at 24 hours postoperative were $78.3 \pm 45$ ng/mL and $119.26 \pm 103.5$ ng/mL in the AKI and non-AKI groups, respectively ($P=0.009$; Table 2).

The area under the receiver operating characteristic curve (AUC-ROC) for plasma NGAL levels after coronary artery bypass graft had a sensitivity of 77% and specificity of 81% in prediction of AKI (the cut-off value equal to 126.4 ng/mL; Figure 1). None of the cases who developed AKI showed grade 3 AKI and no patient had dialysis requirement.

**Discussion**

The current study indicated that the risk of AKI associated with coronary artery bypass graft according to the serum creatinine or plasma NGAL concentration showed a transient acute renal injury. Plasma NGAL is adequate to predict result. NGAL is a 25 kDa protein which bound to matrix metalloproteinase-9 from neutrophils and is expressed in neutrophils and other cell types, such as renal tubular cells, epithelial cells and hepatic cells by inflammatory or injury stimuli. It is also induced in some human cancers (12). Post-operative kidney dysfunction, with or without AKI, has several causes including: nephrotoxin use, a pre-operative lack of fluid, vasoactive agent administration, inadequate cardiac function, declines in renal perfusion during surgery, post-operative lack of fluid, inflammation, and infection. Furthermore, risk factors for post-operative kidney dysfunction are defined diabetes, male gender, age greater than 56 years and hypoalbuminemia (13).

Our inclusion criteria were rather strict, leading to a homogeneous study subjects undergoing planned CABG.

### Table 1. Demographic features between two groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group without AKI No. (%)</th>
<th>AKI group No. (%)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30 (53.6%)</td>
<td>17 (53.6%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Female</td>
<td>26 (46.6%)</td>
<td>6 (46.4%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36 (64.3%)</td>
<td>20 (87%)</td>
<td>0.04</td>
</tr>
<tr>
<td>No</td>
<td>20 (35.7%)</td>
<td>3 (13%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19 (33.9%)</td>
<td>8 (34.8%)</td>
<td>0.94</td>
</tr>
<tr>
<td>No</td>
<td>37 (66.1%)</td>
<td>15 (65.2%)</td>
<td></td>
</tr>
<tr>
<td>Angiotensin-converting enzyme/ or angiotensin receptor blocker administration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (46.4%)</td>
<td>16 (69.6%)</td>
<td>0.06</td>
</tr>
<tr>
<td>No</td>
<td>37 (53.6%)</td>
<td>7 (30.4%)</td>
<td></td>
</tr>
<tr>
<td>Non-steroidal anti-inflammatory drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5 (8.9%)</td>
<td>4 (17.4%)</td>
<td>0.28</td>
</tr>
<tr>
<td>No</td>
<td>51 (91.1%)</td>
<td>19 (82.6%)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Creatinine and NGAL level

<table>
<thead>
<tr>
<th>Variable</th>
<th>AKI group Mean ± standard deviation</th>
<th>Group without AKI Mean ± standard deviation</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>65.91±7.7</td>
<td>63.91±9.8</td>
<td>0.406</td>
</tr>
<tr>
<td>Serum creatinine (first day)</td>
<td>1.23±0.49</td>
<td>1.11±0.27</td>
<td>0.16</td>
</tr>
<tr>
<td>Serum creatinine (second day)</td>
<td>1.56±0.53</td>
<td>1.09±0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine (third day)</td>
<td>1.70±0.5</td>
<td>1.02±0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NGAL before CABG</td>
<td>78.3±45</td>
<td>119.26±103.5</td>
<td>0.54</td>
</tr>
<tr>
<td>NGAL after CABG</td>
<td>207.2±180.8</td>
<td>119.26±103.5</td>
<td>0.009</td>
</tr>
</tbody>
</table>

NGAL, Neutrophil gelatinase-associated lipocalin, CABG, Coronary artery bypass graft.
techniques. The expected risk for post-operative AKI in this procedure is elevated in our group. In this study, AKI as a postoperative complication happened in 23 (29%) cases. AKI was defined according to the AKI criteria by KIDGO in 2012 as a minor change of 0.3 mg/dL serum creatinine level (4). It is proposed that the lack of success of the diagnosis of AKI according to NGAL may be due to the low incidence identified of AKI by decreases in serum creatinine and urine output, subsequently this study identified AKI based on the increase of the serum NGAL and creatinine levels. Also, typical risk factors of AKI evaluated by accessible factors were definite in the study group.

Previous studies support the NGAL as a useful biomarker for the prediction of AKI following cardiopulmonary bypass in a pediatric population (14) and in adults (2-5, 13-17), after coronary angiography and percutaneous coronary intervention (18) as well as transcatheter aortic valve implantation (19). The results confirmed a significant association between a rise in serum NGAL levels postoperatively to the occurrence of post-operative AKI more rapidly than serum creatinine. Furthermore, serum NGAL had an acceptable specificity and sensitivity for diagnosis of AKI, with an acute rise following injury when compared to creatinine. It begins to rise within twelve hours after kidney dysfunction while creatinine rise occurred over 1–3 days. The findings are similar to the previous studies (1,2,15,16,20), suggesting that NGAL can be potentially used as an early biomarker for detection of post-reparative AKI.

The ROC curve of plasma NGAL (with the cut-off value of 126.4 ng/mL) within 12 hours of coronary artery bypass graft showed that NGAL had a specificity of 81% and sensitivity of 77% to predict the kidney injury.

Balkanay et al reported that the AUC-ROC (area under the ROC curve) was 0.96 at the 150 ng/mL cut-off value of the postoperative first-day plasma NGAL concentration (21). Other surveys have demonstrated that the AUC-ROC of the postoperative second hour urinary NGAL were measured between 0.85 and 0.87 to the development of AKI after cardiac surgery. In addition, the AUC-ROC of the plasma NGAL level was 0.64 for detecting AKI (21-23). In our study, blood plasma samples (with available facilities) were taken 12 hours after CABG, while in the previous studies, the levels of NGAL in serum and urine were measured within 2–4 hours. The researchers supposed that the peak timing for NGAL measurements after cardiac surgery was within 2–4 hours or at the time the patient was admitted to the intensive care unit (20). A result of a meta-analysis, the intraoperative discrimination of AKI by urine NGAL was demonstrated as an AUC-ROC <0.70. In the immediate 24-hour postoperative period, the urine NGAL exhibited AUC-ROC of 0.69 to 0.72 and the postoperative plasma NGAL was <0.70 (24). In parallel with these results, we obtained that measurements of the plasma NGAL level within 12 hours postoperative can be used as an early biomarker in AKI detection after CABG with a high sensitivity and specificity.

Since no patient who developed AKI, had grade 3 AKI and neither needed dialysis, the diagnostic value of NGAL levels could be evaluated by defining its predictive usefulness for mortality, progression to dialysis-dependent renal failure or clinical outcomes. Another study conducted by Bulluck et al reported that the preoperative serum NGAL levels can predict postoperative AKI after considering some clinical parameters included gender, age, hypertension, diabetes mellitus, baseline estimated glomerular filtration rate, baseline high sensitivity troponin T, baseline ejection fraction, prior CABG, type of surgery planned and use of intra-aortic balloon pump. Moreover, it was revealed that, the serum NGAL stage was also strong marker of mortality in a subgroup analysis of patients with normal creatinine levels (25). In addition, Bulluck et al proposed that the last tertile of preoperative serum NGAL (>220 ng/L) had an estimated double increase risk of mortality at one year (25). However, the monitoring of serum NGAL values can valuably be for predicting AKI in cases undergoing CABG.

**Conclusion**

Our results recommend that the monitoring of serum NGAL values could be helpful for early diagnosis of AKI in our patients undergoing CABG. However, this finding needs further multicenter studies with larger patient populations.
Limitations of the study
One of the main limitations of this study is small number of patients (n = 79). Furthermore, it is better, monitoring of the serum NGAL values in earlier times of post-operation.

Authors' contribution
Conceptualization: AP, SJ.
Methodology: AP, SJ.
Validation: HMZ.
Formal analysis: KK.
Investigation: NP.
Resources: AE.
Data curation: NP.
Visualization: SJ.
Supervision: AP, SJ.
Project administration: AP.
Funding acquisition: AP, SJ.
Writing—original draft : AP, SJ, NP.
Writing—review and editing: All authors.

Conflicts of interest
The authors declare that they have no competing interests.

Ethical issues
The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Zaman University of Medical Sciences approved this study (Ethical code #IR.ZUMS.REC.1394.02). Accordingly, written informed consent was taken from all participants before any intervention. Accordingly, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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