Neutrophil Gelatinase-Associated Lipocalin as a Marker for Acute Kidney Injury in the Elderly after Operation for Correction of Femur Proximal Fracture

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Abstract

Perioperative acute kidney injury (AKI) is responsible for about 25% of all cases of renal dysfunctions, increasing postoperative mortality. Elderly patients are at higher risk of developing AKI following major surgeries and a marker that could predict its occurrence in the first postoperative hours is still to come.

Objectives: To assess the plasma NGAL as an early postoperative AKI predictor in elderly patients undergoing femoral fracture repair under spinal anesthesia.

Method: Fifty-seven elderly patients undergoing proximal femoral fracture repair were prospectively studied over a period of 48 hours after surgery. Blood samples were collected for analysis of postoperative NGAL, 4 and 24 hours after the procedure. Plasma creatinine measurements were done at hospital admission, then 24 and 48 hours postoperatively. The diagnosis of AKI was made by the RIFLE criteria (Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease) and an increase in the plasma creatinine of at least 1.5 times its baseline values (Risk stage) was considered. The accuracy of the NGAL for the diagnosis of AKI was analyzed according to the area under the Receiving Operating Characteristic curve.

Results: Sixteen (28%) patients developed postoperative AKI. The accuracy of plasma NGAL on the diagnosis of AKI at four and 24 hour was 0.799 (95% confidence interval: 0.663 – 0.936, p <0.001) and 0.805 (95% confidence interval: 0.665 – 0.946, p <0.001), respectively.

Conclusion: Plasma NGAL has good accuracy on the diagnosis of AKI when measured four and 24 hours after femoral fracture repair in the elderly.

ABBREVIATIONS

AKI: Acute Kidney Injury; NGAL: Neutrophil Gelatinase-Associated Lipocalin; RIFLE: Risk, Injury, Failure, Loss And End-Stage Kidney Disease.

INTRODUCTION

Acute kidney injury (AKI) has an overall incidence of 23% in hospitalized patients [1] when using the definitions proposed by the Acute Dialysis Quality Initiative (ADQI) and the modified version proposed by the Acute Kidney Injury Network (AKIN) [2]. Surgeries, especially cardiovascular surgeries, are a leading cause of AKI, ranging from 18% to 47% of all cases of hospital-acquired AKI [3]. Despite non cardiovascular surgical have been less studied compared with cardiovascular surgery, it is also cause of AKI. Thakar et al., (2009) observed that orthopedic surgery is cause of AKI in surgical settings in intensive care units [4]. Elderly patients are at increased risk of developing kidney injury following a major orthopedic surgery. For correction of femur fracture is common to use of nonsteroidal anti-inflammatory for intraoperative analgesia, occurrence of hypotension after spinal anesthesia, blood loss and blood transfusion requirements, and the use of vasopressor bolus, they are all risk factors for AKI [4]. Added to them is the fear of fluid administration in elderly patients. This notion is confirmed in the study by Azevedo et al., (2008) that demonstrated an incidence of 21% of AKI in the postoperative period using RIFLE criteria in patients over 65 years of age who underwent surgery to repair femoral fractures [5].

AKI is an independent risk factor for mortality with rates reaching 60% in patients who require renal replacement therapy [6]. High mortality rates may be associated with the lack of sensitive and specific markers of AKI available in clinical practice, which could enable early diagnosis and treatment, thereby preventing its progression. Creatinine is commonly used for this purpose but fails in many respects, primarily due to its late increase after the beginning of the injury [7]. Urine output is also not a good marker for AKI and is very unspecific for its diagnosis [8]. Many patients in critical condition developed oliguria when admitted to the intensive care unit, but it is not followed by AKI [9]. Thus, a suitable marker to evaluate and diagnose AKI is not currently available.

A possible better marker for AKI might be the NGAL molecule (neutrophil gelatinase-associated lipocalin), which is also known as lipocalin 2. NGAL is a protein that is synthesized in the bone marrow and stored in neutrophil granules [10]. NGAL is present in several types of human tissues, such as in the lungs, liver and kidney [10]. Upon damage to the epithelial tissue of these organs, NGAL gene expression increases, especially when renal tubular epithelial injury occurs [10]. There are three different molecular forms of NGAL: monomeric, dimeric and trimeric [10]. The monomeric form exhibits a molecular weight of 25 kDa and is of particular importance to AKI [10]. Monomeric NGAL is filtered by the kidney glomeruli and is largely reabsorbed thereafter by the proximal tubule. When there is a decrease in glomerular filtration rate (GFR) and damage to the renal tubular cells, NGAL appears in higher concentrations in plasma and urine [10].

NGAL has been studied in surgical and clinical patients. In a recent systematic review, NGAL exhibits good accuracy in patients undergoing cardiac surgery, renal transplantation and clinical patients in critical condition [11]. However, to the best of our knowledge, this marker has not been studied in elderly patients who are undergoing orthopedic surgeries.

Elderly patients (the World Health Organization defines “elderly” as a person over 60 years of age in underdeveloped countries [12]) present important risk factors and often develop AKI [13]. Conditions, such as chronic renal injury, systemic arterial hypertension, mellitus diabetes, atherosclerosis and congestive heart failure, are some of the main diseases that affect this population, thus causing this population to be more likely to develop AKI [14]. Additionally, when undergoing femoral fracture repair surgery, the elderly are exposed to other mechanisms of renal injury, such as hypovolemia, hypotension, acute anemia, rhabdomyolysis, and use of nephrotoxic drugs, such as diuretics and anti-inflammatory [15].

The aim of this study was to evaluate the accuracy of the NGAL molecule by analyzing the ROC curve in elderly patients undergoing proximal femur osteosynthesis.

MATERIALS AND METHODS

Patients

Elderly patients undergoing proximal femur osteosynthesis (including partial and total hip arthroplasty surgery) conducted at a tertiary public hospital in Vitória da Conquista (located in the state of Bahia, Brazil) were eligible for the study. A total of 66 patients were recruited in a prospective unicusentric cohort from February 1 to July 31, 2014. Following approval from the Center of Education and Research and the Ethics Committee of the same hospital (this paper is also registered in the Plataforma Brasil (www.saude.gov.br/plataformabrasil) under the CAAE: 15062014.0.3001.0055) and after obtaining written informed consent from patients or their legal guardians, the accuracy of the NGAL marker as a predictor of AKI was evaluated.

Inclusion criteria were patients aged 60 years and older who were classified as ASA 1 or ASA 2 in the American Society of Anesthesiologists Physical Status Classification System and who were undergoing surgery for proximal femoral fracture repair under spinal anesthesia, which is the anesthetic technique most frequently used at the study site.

The study excluded patients who had chronic kidney injury (CKI) as evidenced by a baseline creatinine value greater than 2.0 mg.dL⁻¹ or were on dialysis and those definitely unsuitable for spinal block (coagulopathy, infection at the puncture site, patient refusal). Patients with malignant neoplasia were also excluded given their high serum NGAL concentrations. Patients with decompensated chronic diseases had surgery postponed until the underlying disease was controlled.

An at least 1.5-fold increase in the value of baseline creatinine (risk stage of the RIFLE classification) was required for the diagnosis of AKI. This information was requested at patient admission and adopted as the baseline creatinine. In cases where surgery was postponed for a period longer than seven days, a new creatinine test was requested and used as a reference.

When suitable for the procedure, patients were referred to the operating room after 8 hours of fasting and without anesthetic premedication. Patients were monitored with continuous ECG in leads DII and V5, noninvasive blood pressure monitoring and pulse oximetry.

Given that it is not common practice, urinary catheterization was not performed, and diuresis was not measured. Peripheral venous access was performed with a 20- or 18-G catheter. Patients underwent standard anesthesia pre-defined by the researcher and the anesthesiology team (subarachnoid block with lumbar puncture to the average level of L2 to L3 or L3 to L4 with Quincke 25-G needle after further cerebrospinal fluid return, separately intrathecal injection of 12 to 15 mg of bupivacaine isobaric chloride and 60 μg of morphine). Anesthesia was performed by different anesthesiologists, and surgical procedures were performed by different orthopedic surgeons.

All patients received antibiotic prophylaxis with 2 g cephalothin in a single intravenous dose and supplementary oxygen via nasal cannula at a flow rate of 3 L.min⁻¹. The intraoperative fluid therapy was performed according to the anesthesiologist’s clinical judgment with regard to volume and the type of expander (crystalloids with saline solution or Ringer lactate, colloid or blood products). However, a volume replacement of at least 10 mL.kg⁻¹.h⁻¹ with crystalloid solutions was intended. Intraoperative hypotension (defined as systolic blood pressure less than 90 mmHg or a reduction of 30% in relation to baseline [16]) was promptly treated with vasopressors (etilefrine hydrochloride or metaraminol bitartrate were most
commonly used). Due to a lack of available ICU beds at the study site, patients were placed in the intensive care unit only if they exhibited hemodynamic instability requiring vasopressor drugs in continuous infusion, hypoxemia requiring additional oxygen to maintain oxygen saturation >90% or alteration in the level of consciousness. Otherwise, patients were sent to the recovery room and later to the ward.

**Sample collection**

A blood sample was collected for the first measurement of plasma NGAL immediately after the end of surgery. Subsequent samples were collected into a tube containing EDTA anticoagulant 4 and 24 hours after the operation. Blood sample were centrifuged at 3000 rpm for 10 min at 4°C. Samples were stored in cryo-tubes and subsequently stored in aluminum canisters (Volta brand CryoSystem series) containing liquid nitrogen at a temperature less than -150°C until further analysis.

**Assays**

Plasma NGAL levels were analyzed using enzyme-linked immunosorvent assay (ELISA; Bioporto, Hellerup, Denmark). For the measurement of creatinine, the study’s methodology is based on the Jaffé method.

**Sample size**

MedCalc software was used to calculate the sample size based on the formula proposed by Hanley and McNeil. An alpha of 0.05 and power of 90% were considered. For an area under the Receiving Operating Characteristic curve (AUROC) of 0.800 and a 25% incidence of AKI, a minimum of 48 patients would be required.

**Statistical analysis**

A descriptive analysis of quantitative variables as means and standard deviations was performed for data exhibiting a normal distribution, and median and quartiles were reported for non-normal and categorical variables through absolute and relative frequencies. In exploratory investigations, creatinine and NGAL values were compared between the groups with or without AKI (at any degree) using Student’s t test for independent samples or Mann-Whitney test for the distribution of the variable. The association between NGAL (independent variable) and AKI (dependent variable) was assessed using the Poisson regression technique. Univariate and multivariate (adjusted for hypotension, age, comorbidities and the occurrence of hypotension) robust models were calculated to estimate the relative risk (RR) and respective 95% confidence intervals (95%CI).

To evaluate the accuracy of the NGAL molecule in the prediction and/or diagnosis of AKI, the discriminatory capacity was analyzed through AUROC and its calibration using the Hosmer-Lemeshow test. The best cutoff point was reported. Additionally, after dichotomization of NGAL values greater than/equal to or less than 150 ng.mL⁻¹, contingency tables were compiled with calculation of the respective sensitivity, specificity, positive and negative predictive values as well as positive and negative likelihood ratios with respective confidence intervals at 95%.

All tests were two-tailed, and final results with p ≤ 0.05 were considered to be statistically significant.

Data were analyzed using the Statistical Package for Social Sciences software (SPSS, version 20.0 USA).

**RESULTS AND DISCUSSION**

Of the 66 patients eligible for the study, NGAL serum was successfully analyzed in 57 patients. Nine patients were excluded: one patient presented with fever and tachycardia in the operating room, four patients were lost to follow-up and four patients had missing data (Figure 1). Among the remaining patients, 16 (28%) developed AKI in the postoperative period. The clinical characteristics and incidence of acute kidney injury are presented in Table 1.

Intraoperative data were compared between the two groups (with and without AKI) and are presented in Table 2. An increased incidence of hypotension was noted in patients presenting AKI.

Serum levels of plasma NGAL exhibited statistically significant differences at 4 and 24 hours after surgery, and increased concentrations were observed in patients who developed AKI (Table 3). Plasma NGAL levels at the end of surgery were not associated with AKI development. However, NGAL blood concentrations 4 and 24 hours after surgery were positively associated with AKI (Table 4). Multivariate regression models indicated that regardless of the occurrence of hypotension and vasopressor use, the risk of AKI increased by 0.7% and 0.6% for each 1 ng.mL⁻¹ increase in NGAL levels at 4 and 24 hours, respectively.

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) and reasons for positive and negative likelihood of NGAL at three periods (soon after the end of surgery, 4 and 24 hours later) were calculated as shown in Table 5.

AUROC values at three periods and the best cutoff points for NGAL molecule to discriminate AKI are presented in Figure 2.

Although elderly patients are at an increased risk of developing AKI when undergoing surgery for femur fracture correction, the NGAL biomarker has been poorly studied in this population. This study demonstrates a 28% incidence of AKI in postoperative hip fracture in patients older than 60 years of age. Based on RIFLE criterion, an increase in the incidence of intrahospital AKI has been observed [17].

Greater than half of the patients (58%) exhibited systemic hypotension, and this condition was more frequent in patients who developed AKI. The low renal perfusion triggered by hypotension may represent a risk of injury. Elderly patients who are hypertensive (56% of the patients in this study) do not tolerate periods of hypotension as it leads to organ ischemia. In a prospective observational study of patients undergoing major non-cardiac surgery, an analysis with multiple variables indicated that age, comorbidities and the occurrence of hypotension during surgery are independent risk factors for mortality when considering a period of one year after surgery [18]. Therefore, this study argues for tighter control of hemodynamic parameters using invasive blood pressure monitoring, urinary catheterization
Table 1: Clinical characterization of sample and incidence of acute kidney injury (n=57).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± standard deviation)</td>
<td>78.2 ± 9.9</td>
</tr>
<tr>
<td></td>
<td>62.7 ± 12.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6 (10.5)</td>
</tr>
<tr>
<td>2</td>
<td>51 (89.5)</td>
</tr>
<tr>
<td>ASA</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6 (10.5)</td>
</tr>
<tr>
<td>2</td>
<td>51 (89.5)</td>
</tr>
<tr>
<td>Use of NSAIDs</td>
<td>7 (12.3)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>32 (56.1)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (12.3)</td>
</tr>
<tr>
<td>Chronic Obstructive Pulmonary Disease</td>
<td>4 (7.0)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>2 (3.5)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Dementia</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>16 (28.1)</td>
</tr>
<tr>
<td>Risk</td>
<td>6 (10.5)</td>
</tr>
<tr>
<td>Injury</td>
<td>10 (17.5)</td>
</tr>
</tbody>
</table>

Values are presented as n (%), except when specified.

Abbreviations: ASA: American Society of Anesthesiologists physical status classification system; NSAIDs: Anti-inflammatory non-steroidal drugs.

and continuous measurements of urine output. More aggressive volume replacement and early use of vasopressors for treatment of intraoperative hypotension are prophylactic measures for AKI.

AKI was associated with increased plasma concentrations of NGAL 4 and 24 hours after surgery, and statistically significant differences were noted. In this study, the 24-hour NGAL measurement presented a better AUROC. These findings suggest the precocity of the NGAL molecule as a biomarker of AKI compared with creatinine (the latter only exhibited increased serum levels after 48 hours postoperatively), which confirms the hypothesis of the study. Mishra et al., (2005) demonstrated in a population of 71 pediatric patients undergoing cardiac surgery with cardiopulmonary bypass that NGAL increased two hours after the beginning of extracorporeal circulation [19]. Given the early nature of the increase, levels were studied at different times. Perhaps NGAL should be requested at regular intervals to track the evolution of the disease if it is not cost prohibitive given the similarity with tendency of lactate.

In a meta-analysis published in 2009, both plasmatic and urinary NGAL presented a good correlation with the development of AKI. Serum concentrations of 100-279 ng.mL⁻¹ with a mean value of 170 ng.mL⁻¹ for adults were necessary to achieve adequate sensitivity and specificity [20]. The 150-ng.mL⁻¹ value has been used as the cutoff point for the currently available kits. Regarding this value, this study observed high sensitivity but low specificity in all three time points. Despite a wide confidence interval, its low negative likelihood ratio of a concentration less than 150 ng.mL⁻¹ suggests that this marker could be used as an exclusion test for AKI. This study suggests that increased NGAL values should be used as cutoff points.

NGAL concentrations greater than 150 ng.mL⁻¹ were frequently observed during the postoperative period even in patients without AKI. It is unknown whether the presence of the NGAL molecule in this scenario indicates subclinical AKI (not evidenced by creatinine), an inflammatory response marker, or even simply a reduction in the filtration of this molecule motivated by a reduction of GFR, which is a natural aging process. A commercially available NGAL assay, such as the one used in this study, cannot distinguish the molecular form of NGAL, which originates in the kidney as different molecular forms given that circulating neutrophils are released in response to liver and lung inflammation. Haase et al., (2011) suggested that patients with increased subclinical NGAL levels exhibited a poor prognosis [21]. In addition, in an earlier stage of the LRA, direct injury to the renal parenchyma is present and damaging. However, this condition does not cause loss of kidney function. This period represents the best opportunity for early intervention, as it would prevent disease progression [22]. This issue along with a reconsideration of the cutoff point for AKI according to population profile must be further studied.

Uni- and multivariate regression analyses indicated that the continuous variable was a strong independent predictor of AKI. We observed that the RR increased by 0.7% for each 1-ng.mL⁻¹ increase in the NGAL concentration after 4 hours of operation. For instance, a patient who exhibits an increase in their baseline concentration from 100 to 200 ng.mL⁻¹ in the postoperative period presents a 70% risk of developing AKI. Therefore, the association between NGAL and AKI allows us to estimate the risk of developing this disease whenever serial measurements of this molecule are possible.

This study considered the plasma concentration of NGAL molecule immediately at the end of the operation as baseline. However, this concentration dies not truly correspond to the
Figure 2 Accuracy of NGAL according to time of surgery in diagnosis of any acute kidney injury by RIFLE criteria (n = 56).

Table 2: Intraoperative variables according to the absence or the presence of acute kidney injury.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No acute kidney injury (40)</th>
<th>Acute kidney injury (16)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g.dL^{-1})</td>
<td>9.6 ± 1.7</td>
<td>10.0 ± 1.8</td>
<td>0.462</td>
</tr>
<tr>
<td>Crystalloid (mL)</td>
<td>1612.5 ± 744.5</td>
<td>1812.5 ± 543.9</td>
<td>0.335</td>
</tr>
<tr>
<td>Packed red blood cells (mL)</td>
<td>397.0 ± 135.6</td>
<td>318.6 ± 32.9</td>
<td>0.333</td>
</tr>
<tr>
<td>Length of surgery</td>
<td>96.2 ± 42.9</td>
<td>112.8 ± 40.0</td>
<td>0.190</td>
</tr>
<tr>
<td>Total volume infused (mL.kg^{-1}.h^{-1})</td>
<td>17.7 ± 6.1</td>
<td>16.4 ± 6.6</td>
<td>0.332</td>
</tr>
<tr>
<td>Hypotension, n (%)</td>
<td>20 (50.0)</td>
<td>13 (81.2)</td>
<td>0.032</td>
</tr>
<tr>
<td>Use of vasopressor, n (%)</td>
<td>19 (47.5)</td>
<td>13 (81.2)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± standard deviation, except when specified. In one patient, a colloid volume of 500 mL was used. This patient did not present kidney injury.

Table 3: NGAL and creatinine values according to postoperative time and presence of acute kidney injury.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No acute kidney injury (41)</th>
<th>Acute kidney injury (16)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGAL (mean ± standard deviation) ng.mL^{-1}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hour (end of surgery)</td>
<td>216.7 ± 124.6</td>
<td>261.8 ± 102.0</td>
<td>0.204</td>
</tr>
<tr>
<td>4 hours</td>
<td>201.7 ± 96.3</td>
<td>320.1 ± 105.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 hours</td>
<td>195.4 ± 88.6</td>
<td>339.3 ± 130.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine (median and quartiles) mg.dL^{-1}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative</td>
<td>0.9 (0.7 – 1.0)</td>
<td>0.8 (0.7 – 1.1)</td>
<td>0.767</td>
</tr>
<tr>
<td>24 hours</td>
<td>0.9 (0.7 – 1.0)</td>
<td>1.2 (0.7 – 1.7)</td>
<td>0.074</td>
</tr>
<tr>
<td>48 hours</td>
<td>0.8 (0.7 – 1.0)</td>
<td>1.4 (1.2 – 2.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NGAL baseline concentration. The NGAL measurement was chosen immediately after the end of the operation to avoid the possibility that intraoperative interventions, such as excessive hydration, interfered with future comparisons between the concentrations of this marker.

Another limitation of this study was the exclusion of patients considered to have CKD with creatinine values greater than 2 mg.dL^{-1}. CKD is an important risk factor for AKI in the perioperative period. Furthermore, patients with CKD exhibit increased baseline values for NGAL serum [23]. Studies evaluating
In this work, the NGAL molecule was compared with creatinine, which does not function well in standard diagnostic tests. In addition, due to the lack of knowledge regarding the creatinine baseline of patients, reference measurements were obtained at hospital admission. However, at this time point, creatinine level could have been altered and thus not correspond to basal creatinine levels.

Given the short follow-up period, this study was not designed to assess whether NGAL is related to outcomes. However, a recent systematic review of different populations revealed that high serum and urinary NGAL concentrations are associated with the severity and duration of the AKI. Both levels can be increased during hospital and ICU stay, which also increases the need for renal replacement therapy and mortality [24,25].

CONCLUSION

This study demonstrated that the plasma NGAL biomarker is a good predictor of AKI when measured 4 and 24 hours after surgery in elderly patients who underwent proximal femur fracture correction. However, cutoff points are greater than 150 ng.mL\(^{-1}\).

REFERENCES


