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# Whole blood neutrophil gelatinase–associated lipocalin predicts acute kidney injury in burn patients

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## Abstract

**Background—**Early detection of acute kidney injury (AKI) in severely burn-injured patients can help alter treatment to prevent progression to acute failure and reduce the need for renal replacement therapy. We hypothesized that whole blood neutrophil gelatinase –associated lipocalin (NGAL) will be increased in severely burn-injured patients who develop AKI during acute resuscitation.

**Materials and methods—**We performed a prospective observation study of adult burn patients with a 20% total body surface area (TBSA) burned or greater burn injury. Two-hour serial measurements of NGAL, serum creatinine (Cr), and hourly urine output (UO) were collected for 48 h after admission. Our primary goal was to correlate the risk of AKI in the first week after burn injury with serial NGAL levels in the first 48 h after admission. Our secondary goal was to determine if NGAL was an earlier independent predictor of AKI compared with Cr and UO.

**Results—**We enrolled 30 adult (age ≥ 18 y) burn patients with the mean ± standard deviation age of 40.9 ± 15.4 and mean TBSA of 46.4 ± 22.4. Fourteen patients developed AKI within the first 7 d after burn injury. There were no differences in age, TBSA, fluid administration, mean arterial pressure, UO, and Cr between AKI and no-AKI patients. NGAL was significantly increased as early as 4 h after injury (182.67 ± 83.3 *versus* 107.37 ± 46.15) in the AKI group. Controlling for age, TBSA, and inhalation injury, NGAL was a predictor of AKI at 4 h after injury (odds ratio, 1.02) and remained predictive of AKI for the period of more than the first 24 h after admission. UO and Cr were not predictive of AKI in the first 24 h after admission.

**Conclusions—**Whole blood NGAL is markedly increased in burn patients who develop AKI in the first week after injury. In addition, NGAL is an early independent predictor of AKI during



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Author contributions: Soman Sen and Nam Tran designed the experimental protocol. Zack Godwin and Amanda Steele screened and consented patients. Zack Godwin and Amanda Steele also collected all the data. Tina Palmieri and David Greenhalgh were involved in the experimental design and editing drafts of the manuscript. Soman Sen analyzed the data and wrote the drafts of the manuscript. Nam Tran also edited drafts of the manuscript.

**Disclosure**

The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in the article.

acute resuscitation for severe burn injury. UO and Cr are not predictive of AKI during this time period.

**Keywords**

Acute renal injury; Burn injury; NGAL; Resuscitation

## 1. Introduction

Acute kidney injury (AKI) in critically ill burn patients is a morbid and often fatal complication. The prevalence of AKI in severely burn-injured patients is reported to be as high as 53% with a mortality rate >30% [1]. Current methods of evaluating renal function rely on serum creatinine (Cr) measurements and hourly urine output (UO). However, these measurements are often insensitive to rapid changes in renal function because of acute injury [2]. Serum Cr has a long half-life (3.85 h) and is subject to variations because of muscle mass, age, and gender, thus limiting its effectiveness as a sensitive marker of rapid changes in renal function [3]. Decreasing UO may reflect AKI because of reduced glomerular filtration; however, neurohormonal and functional changes influence diuresis and thus UO may be normal despite ongoing renal injury [4].

Neutrophil gelatinase–associated lipocalin (NGAL) is a molecule that is released by polymorphonuclear granulocytes during inflammation and is uniquely produced by endothelial cells of nephrons for urinary secretion and subsequent reabsorption into bloodstream [5]. Under normal conditions, low amounts of NGAL circulate in the bloodstream and are freely filtered by the glomerulus. Normal reference intervals have been suggested of 40–100 ng/mL [6]. During AKI, NGAL rapidly accumulates in the serum because of an increase in secretion by the nephrons and reduction in glomerular filtration rate [7]. This, coupled with a short half-life (10 min) makes whole blood NGAL a potential sensitive and early marker of AKI.

In adult critically ill medical patients, increased serum NGAL levels (155 nmol/L) showed high sensitivity and specificity for AKI. Moreover, NGAL was increased 48 h before significant changes in Cr and UO [8]. In addition, serum NGAL improves the accuracy of AKI predictions compared with Cr alone [9,10]. NGAL also may be a more sensitive predictor of risk for AKI compared with standard methods. A large pooled study of more than 1200 critically ill patients indicates that NGAL is able to detect patients with AKI before any changes in Cr occur [11]. Serum NGAL may also be a marker of renal recovery. A study of 181 patients showed that NGAL levels decreased in the plasma in patients who recovered from AKI [12].

In burn patients, NGAL is also an early marker for AKI. In a study of 45 adult burn-injured patients, NGAL was measured three times after admission (on admission, day 3, and day 7 after burn injury) and found that NGAL levels at days 3 and 7 were significantly higher in patients who developed AKI [13]. Of note, 27% of the patients in this study developed AKI in the second week after injury. In a study of 22 pediatric burn patients, both urine and serum NGAL levels were increased both on admission and on the fifth day after burn injury in patients with AKI. However, Cr was not different at either time point [14].

We hypothesized that after severe burn injury, an increased NGAL level within the first 12 h after injury would be associated with the development of AKI in the first week after burn injury. Our primary aim was to prospectively measure serial serum NGAL, serum Cr, and hourly UO during the first 48 h after burn injury and correlate these measurements to the development of RIFLE (risk, injury, failure, loss, end-stage)-based criteria for AKI in the first 7 d after injury [15]. Our secondary aim was to determine if NGAL was a more rapid and responsive marker of AKI compared with serum Cr and hourly UO.

## 2. Methods

A prospective observational study was conducted for adult burn-injured patients who were admitted to our regional burn center. Inclusion criteria included age 18 y or older, 20% or greater total body surface area (TBSA) second and third degree burn injury, and admission to our burn center within 2 h of burn injury. Patients were excluded from the study if they had a nonsurvivable burn injury or a history of preinjury renal disease. A nonsurvivable burn injury was determined at the time of admission by the attending burn surgeon and determination of preinjury renal disease was made through interrogation of the patient's past medical history. All patients were admitted to the burn intensive care unit and standard burn resuscitation protocol was started under the direction of the attending burn surgeon. The University of California Davis Institutional Review Board approved all protocols for the study.

After enrollment, serial whole blood NGAL and Cr measurements were collected every 2 h for the first 48 h after admission. NGAL and Cr were measured from 1.5 mL blood samples using the point of care Triage System (Alere, San Diego, CA) and The StatSensor Creatinine meter (Nova Biomedical, Waltham, MA). Both the Triage System NGAL and The StatSensor Creatinine results were calibrated to the central hospital clinical laboratory's UniCel DxC 800 Synchron chemistry analyzer (Beckman Coulter, Brea, CA) before the initiation of the study. In addition to serial measurements of Cr and NGAL, hourly heart rate, respiratory rate, mean arterial pressure, central venous pressure, fluid intake, and UO were recorded for 48 h. Research personnel recorded all point of care assays and results. The treating burn care team, including the physicians and nurses, was blinded to the results of the serial NGAL and Cr measurements. AKI was diagnosed based on renal RIFLE criteria [16]. Patients were considered as having AKI if they met the "Injury" level of the RIFLE criteria in the first 7 d after burn injury. Injury is defined as UO of 0.3 to <0.5 mL/kg/h for at least 12 h, a doubling of the serum Cr from baseline, or a decrease in glomerular filtration rate of >50% but <75%. Failure is defined as a serum Cr rise three times of baseline or a UO <0.3 mg/kg/h.

R statistical package (www.r-project.org) was used to analyze the data. Continuous variable comparisons between two groups were performed using the two-sample Student *t*-test for continuous data. Times series data (e.g., serial measurements) were analyzed using repeated measures analysis of variance. *Post hoc* analysis was performed using pairwise comparisons with a Bonferroni adjustment for significant repeated measures analysis of variance results. The Fisher's Exact test was used to assess association between discrete categorical variables. Multivariate logistic regression analysis was performed to determine associations between

categorical outcome variables and independent continuous and categorical predictor variables. Receiver-operator characteristic curves were generated and area under the curve was calculated for NGAL, Cr, and UO 4 h after admission. Multivariate logistic regression analysis was performed for the development of AKI in the first week of admission (categorical variable). Initial variables for multivariate logistic regression were selected based on their clinical relevance as documented in the literature. Studies have shown burn size, inhalation injury, and age as predictors of outcome in severely burned patients. A stepwise (backward) approach was used for the development of the multivariate model. Predictor variables for the analysis included NGAL, Cr, hourly UO, age, gender, TBSA, and inhalation injury. Statistical significance was set at a *P* value of <0.05.

## 3. Results

A total of 30 patients were enrolled in the study, 25 men and five women. Mean age for the entire sample was 41 ± 15 y and mean TBSA was 46 ± 22%. For the period of more than the first 48 h after injury, mean NGAL was 143 ± 75 ng/mL, mean Cr was 1.07 ± 0.4 mg/dL, and mean hourly UO was 1.04 ± 0.7 mL/ kg/h. AKI was diagnosed in 14 (46.7%) patients in the first week after burn injury with average time to diagnosis of 28 ± 17 h. Three of these patients progressed to require dialysis; however, none of these patients required dialysis in the first week after injury.

### 3.1. AKI versus no AKI

There was no significant difference in age, TBSA, mean hourly fluid rate, mean arterial pressure, central venous pressure, and hourly UO between AKI and no-AKI patients (Table 1). Mean NGAL for the period of more than the first 48 h after injury was significantly higher in AKI patients (185 ± 86 ng/mL) than no-AKI patients (112 ± 48 ng/mL). However, blood urea nitrogen and serum Cr were not significantly different between the AKI and no-AKI groups (Table 2). NGAL was significantly higher at 4 h after admission in the AKI group (181 ± 104 ng/mL) compared with the no-AKI group and remained significantly increased for the period of more than the first 24 h after admission. However, Cr and hourly UO were not significantly different at any time point between 0 and 24 h after admission between the AKI and no-AKI groups (Table 2).

### 3.2. NGAL predicts AKI early during acute resuscitation

Multivariate logistic regression analysis was performed to determine if at any time point within the first 24 h after admission, NGAL, Cr, and UO were predictive independent variables for the development of AKI in the first 7 d after injury. The regression models were controlled for age, TBSA, and the presence of inhalation injury. At 4 h after injury, NGAL was predictive of AKI (odds ratio [OR], 1.02 (1.004–1.04)), however, Cr and UO were not predictive (Table 3). Receiver-operator characteristic curves were generated for NGAL, Cr, and UO 4 h after admission. The area under the curve for NGAL was 0.82 (95% confidence interval [CI], 0.65–1), Cr was 0.55 (CI, 0.29–0.82), and UO was 0.65 (CI, 0.44–0.86) (Fig. 1). At both 12 h (OR, 1.03 [1.01–1.06] and 24 h (OR, 1.02 [1.002–1.04] after injury, NGAL remained predictive of AKI; however, at no time point within the first 24 h after admission were Cr or UO predictive of AKI (Table 3).

## 4. Discussion

Our study differs from the previous studies in burn patients in several ways. First, we performed a serial comparison of serum NGAL with both UO and serum Cr for the first 48 h after injury. Second, our overall burn severity was higher reflecting a higher risk population for AKI. This is reflected in the fact that 47% of our patients developed AKI, which is in line with previously published results [1]. Third, we also compared other parameters during resuscitation that may impact AKI development such as fluid intake. Our findings are similar to published reports in both burn injury and critically ill populations. We found that whole blood NGAL is markedly increased in burn patients with AKI before any significant changes in Cr. We also found that significantly increased NGAL at 4 h after admission for burn injury independently increases the risk of AKI but that neither increased Cr nor decreased UO is predictive of AKI in the first 24 h after burn injury. We do acknowledge, however, that there is a cross-sectional nature between AKI and NGAL. Because increased whole blood NGAL may reflect ongoing renal injury and thus increased NGAL could also occur as a result of AKI. In this study, NGAL was significantly increased at 4 h after admission; however, based on RIFLE criteria, none of the patients could be identified as having AKI at this time point. Thus, the use of RIFLE limits the identification of patients with AKI up to 12 h after admission. An increased whole blood NGAL may have value in identifying patients with AKI at an earlier time point.

It is possible that other forms of serum NGAL are more specific for AKI. Serum NGAL is secreted under stress conditions in dimeric form from several sources in the body including epithelial cells, tumor cells, liver, and kidney [17]. However, the monomeric form of NGAL is only secreted from renal tubular cells and is specific to the kidney [18]. Current available clinical assays for whole blood NGAL do not distinguish between the monomeric and dimeric forms. In correlation with this, several studies suggest that whole blood NGAL is not as sensitive a marker for AKI as urinary NGAL [19,20]. Additionally, whole blood NGAL may not perform any better than standard RIFLE criteria for predicting mortality for AKI patients [21]. Despite this limitation, we believe that whole blood NGAL has value as an early biomarker for AKI in burn patients. In our study, whole blood NGAL was increased over the upper limit of the reference value (100 ng/mL) in both AKI and no-AKI patients. This probably reflects the increased NGAL secretion from other sources such as liver and epithelial cells. AKI patients, however, had an earlier and markedly increased level of NGAL (Fig. 2), mostly likely because of decreased glomerular filtration of NGAL and increased secretion by renal tubular cells. This increase in serum NGAL reflects both decreased kidney function and increased kidney damage in AKI burn patients. Moreover, increased serum NGAL independently predicted AKI in the first 4 h after admission and is a better early predictor of AKI compared with serum Cr and UO.

## 5. Conclusions

Whole blood NGAL is increased in burn patients who develop AKI in the first few hours after burn injury and is independently predictive of AKI. Serum Cr and hourly UO are not significantly different in the first 24 h in burn patients who develop AKI in the first week after burn injury and neither one is predictive of AKI at any time point in the first 24 h after

burn injury. Further studies are warranted to examine if urinary NGAL or a combination of novel urinary and serum renal specific biomarkers are better predictors of AKI both during acute resuscitation and recovery from burn injury.

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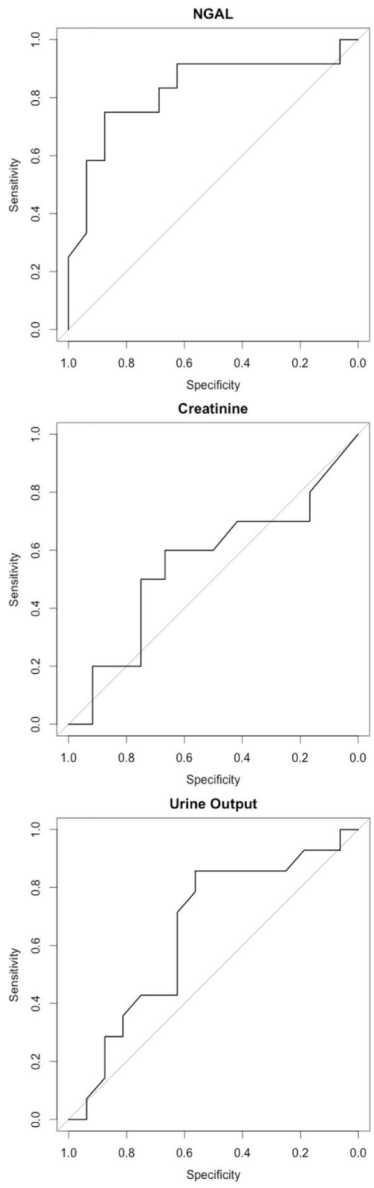


**Fig. 1.**

Receiver-operator curves for accuracy to predict AKI 4 h after admission. Area under the curve (AUC) for NGAL 4 h after admission is 0.82 (CI, 0.65–1), AUC for creatinine at 4 h is 0.55 (CI, 0.29–0.82), and the AUC for urine output at 4 h is 0.65 (CI, 0.44–0.86). AKI, acute kidney injury; CI, confidence interval; NGAL, neutrophil gelatinase–associated lipocalin.

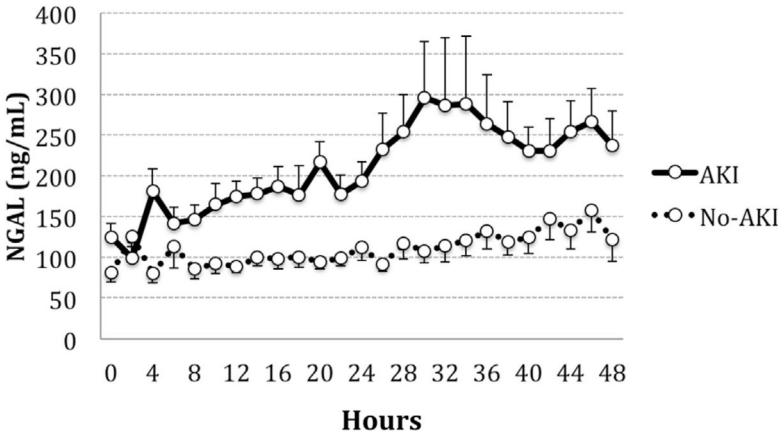


**Fig. 2.**

All values are expressed as the mean ± standard error. AKI, acute kidney injury; NGAL, neutrophil gelatinase–associated lipocalin.

## Table 1

No AKI *versus* AKI patient demographics.

| Patient demographic | No AKI | AKI | *P* value |
|---|---|---|---|
| Patients | 16 | 14 | |
| Age (y) | 41.3 ± 15 | 40.36 ± 16.5 | 0.79 |
| TBSA (%) | 43.67 ± 20.1 | 49.67 ± 25 | 0.46 |
| 24 h Fluid (mL/kg/TBSA) | 3.5 ± 1.4 | 2.9 ± 1.2 | 0.22 |
| MAP | 82.53 ± 5.6 | 79.55 ± 8.3 | 0.25 |
| CVP | 13.4 ± 5.5 | 14.9 ± 3 | 0.37 |
| UO (mL/kg/h) | 1.12 ± 0.96 | 0.96 ± 0.4 | 0.52 |
| NGAL (ng/mL) | 107.37 ± 46.15 | 182.67 ± 83.3 | 0.004 |
| Creatinine (mg/dL) | 0.94 ± 0.16 | 1.23 ± 0.54 | 0.051 |
| BUN (mg/dL) | 10.3 ± 3.4 | 13.7 ± 4.4 | 0.08 |
| Dialysis | 0 | 3 | 0.09 |

AKI = acute kidney injury; BUN = blood urea nitrogen; CVP = central venous pressure; 24 h Fluid = total fluid administered in the first 24 h after admission; MAP = mean arterial pressure; NGAL = neutrophil gelatinase–associated lipocalin; TBSA = total body surface area; UO = urine output.

All mean values are expressed as the mean ± standard deviation for the period of more than the first 24 h after admission.

## Table 2

Serial hourly comparisons of NGAL, creatinine, and urine output in no-AKI *versus* AKI groups.

| Serial NGAL levels | No AKI | AKI | *P* value |
|---|---|---|---|
| NGAL (ng/mL) | | | |
| Hour 4 | 80.25 ± 45.9 | 181.2 ± 104.9 | 0.002 |
| Hour 12 | 89 ± 31 | 174.8 ± 70.6 | 0.0002 |
| Hour 24 | 112.1 ± 64.11 | 193.69 ± 88.67 | 0.008 |
| Creatinine | | | |
| Hour 4 | 0.94 ± 0.38 | 1.00 ± 0.4 | 0.72 |
| Hour 12 | 0.94 ± 0.36 | 1.13 ± 0.69 | 0.44 |
| Hour 24 | 0.81 ± 0.24 | 1.12 ± 1.03 | 0.31 |
| Urine output (mL/kg/h) | | | |
| Hour 4 | 1.1 ± 1.3 | 0.61 ± 0.61 | 0.19 |
| Hour 12 | 1.02 ± 0.74 | 0.58 ± 0.68 | 0.11 |
| Hour 24 | 1.12 ± 0.8 | 1.10 ± 1.1 | 0.96 |

AKI = acute kidney injury; NGAL = neutrophil gelatinase– associated lipocalin.

Hour designation represents the mean values measured at those time points.

## Table 3

Multivariate logistic regression analysis for prediction of AKI.

| AKI predictor | Odds ratio | 95% CI |
|---|---|---|
| Hour 4 NGAL (ng/mL) | 1.02 | 1.004–1.04 |
| Hour 4 creatinine | 1.11 | 0.10–12.4 |
| Hour 4 urine output (mL/kg/h) | 0.1 | 0.7–1.1 |

AKI = acute kidney injury; CI = confidence interval; NGAL = neutrophil gelatinase–associated lipocalin.

Hour designation represents the mean values measured at those time points.