Original Article

# Evaluation of plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) as an early diagnostic marker of acute kidney injury (AKI) in critically ill trauma patients

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## Abstract

**Background and Aims:** Acute kidney injury (AKI) is a frequent complication of severe trauma associated with high mortality. The aim of this study was to evaluate the diagnostic ability of plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of AKI assessed by RIFLE criteria as reference in trauma patients in intensive care unit (ICU).

**Material and Methods:** This was a prospective observational study. Four hundred and eighteen patients admitted in the trauma ICU with age ≥18 years without known renal diseases were followed-up (serum creatinine, urine output, and estimated glomerular filtration rate) for 5 consecutive days. As per RIFLE criteria, 70 patients were broadly classified as AKI and rest of the patients (n = 348) as non-AKI. Plasma and urine samples of AKI (n = 70) and non-AKI (n = 70) patients were further assessed for 3 consecutive days following admission.

**Results:** Mean plasma NGAL (pNGAL) was significantly elevated in AKI patients as compared with non-AKI patients; on admission: 204.08 versus 93.74 ng/mL ($P = 0.01$); at 24 h: 216.73 versus 94.63 ng/mL ($P = 0.01$); and 48 h: 212.77 versus 86.32 ng/mL ($P = 0.01$). Mean urine NGAL (uNGAL) at 48 h was also significantly elevated: 15.45 ng/mL in AKI patients as compared with 13.48 ng/mL in non-AKI patients ($P = 0.01$). Plasma and urine NGAL levels were significantly associated with increased mortality.

**Conclusion:** pNGAL had good predictive value on admission (area under the receiver operative characteristic [AUROC] 0.84), at 24 h (AUROC 0.88) and 48 h (AUROC 0.87), while uNGAL had moderate performance at 24 h (AUROC 0.61) and 48 h (AUROC 0.71). pNGAL can be used as an early and potent diagnostic and predictive marker of AKI and mortality in critically ill trauma patients.

**Keywords:** AKI, ICU, NGAL, RIFLE criteria, trauma

## Introduction

Acute kidney injury (AKI) is a frequent and fatal complication in trauma patients and carries high morbidity and mortality if the diagnosis is delayed.[1,2] In the intensive care unit (ICU) population, AKI is common with an incidence of 1% to 25%, depending on the criteria used for definition, and is associated with 50% to 70% mortality.[2,3] The presence of even mild AKI is associated with an almost two-fold increase in ICU

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mortality as well as greater duration of mechanical ventilation and length of stay in the ICU.[4] Etiology of AKI in critically ill inpatient is multifactorial. AKI is commonly defined as a reduction in urine output, creatinine clearance, and increase in serum creatinine.[4] However, this often leads to delay in diagnosis and failure to intervene at an earlier stage. Rise in serum creatinine occurs when a significant amount of renal function has already been lost and multiple factors such as age, gender, ethnicity, dietary protein intake, muscle mass or metabolism, hydration status, and drugs can alter serum creatinine levels. Moreover, in hypercatabolic state, as in trauma ICU patients, its levels become unreliable. Hence, there is a need for early and accurate biomarker to diagnose AKI in this setting.

Various biomarkers such as urinary tubular enzymes, urinary low molecular weight proteins, kidney injury molecule-1, urinary interleukin-18, and neutrophil gelatinase-associated lipocalin (NGAL) have been studied in different clinical settings to diagnose early AKI.[5–10] However, due to lack of supportive studies, these biomarkers are not used routinely in clinical practice. NGAL, an iron-transporting protein rapidly accumulating in the kidney tubules and urine after nephrotoxic and ischemic insults, has been proposed as an early, sensitive and noninvasive biomarker for AKI. NGAL is a part of the lipocalin protein family and is a 21-kDa low molecular weight protein secreted by various types of human cells, which include not only activated neutrophils but also other tissues such as the kidneys and cells of the gastro-intestinal and respiratory tracts.[11] NGAL is expressed at low concentrations in healthy human tissues such as kidney, trachea, lung, stomach, small intestine, and colon, but its synthesis may be markedly upregulated in case of epithelial cell injury in the colon, liver, and lung, and especially in the kidney.[12] By virtue of its small size, NGAL is freely filtered by the renal glomeruli without being reabsorbed and can therefore be measured in the urine. Renal expression of NGAL is dramatically increased after kidney injury and NGAL is released in both urine and plasma.[13]

NGAL as a potential early biomarker of AKI has been studied in varied clinical conditions.[14,15] A recently performed meta-analysis,[16] which included 15 studies, suggested NGAL as a good predictor for diagnosis of AKI, the need for renal replacement therapy (RRT), and mortality. These studies, however, have limitations such as the inclusion of patients with preexisting renal disease and use of non-standardized methods of measurements. A high-quality study was therefore required to assess the utility of NGAL in the critical care setting. Hence, we planned a prospective observational study, with the primary objective to sequentially evaluate plasma NGAL (pNGAL) and urine NGAL (uNGAL) as an early diagnostic marker of AKI (assessed by RIFLE criteria) in trauma patients admitted in ICU without any preexisting renal disease prior to admission. The secondary objectives were:

(i) To determine cut-off values of plasma and urine NGAL for various stages of AKI and
(ii) To study the association of NGAL level with ICU and hospital length of stay and mortality.

## Material and Methods

This was a prospective observational study conducted from August 2014 to January 2017 at our institute after approval by the Institutional Ethics Committee. All patients aged 18 to 65 years admitted in ICU within 24 h of trauma were screened for inclusion. Exclusion criteria were inability to obtain informed consent from patient or relative, admission >24 h after injury, history of previous renal transplantation, chronic kidney disease (CKD), known medical history with serum Cr >1.4 mg% on admission, known or suspected ongoing preoperative acute renal failure due to any cause, attributed to pre-renal, intrinsic or post-renal etiologies [with evident history, clinical presentation, ultrasonography, proteinuria, (protein/creatinine ratio >0.5 in random urine sample) and active urinary sediments, increasing serum creatinine or pre-operative oliguria], patients with history of ongoing dialysis, known or suspected renal ischemic insult (such as cardiac arrest) or nephrotoxic insult (other than intravascular contrast procedure) prior to admission, patients presenting to ICU with cardiac arrest before or during enrollment, patients with less probability of survival >24 h, transferred from other hospital and traumatic brain injury with Glasgow Coma Scale (GCS) score of 3. All the patients with any direct injury to kidney/ureter/bladder/renal artery/vein or even suspicion were excluded. During the course of study, those patients or the relative who wished to withdraw from the study at a later stage were also excluded from the study.

To estimate sensitivity (80% with absolute precision as 10%) and specificity (80% with absolute precision as 10%) of NGAL with reference to AKI, with 95% confidence interval (CI), 70 patients with no AKI were required (sensitivity and specificity were derived from previous studies). On the basis of our previous experience, the frequency of AKI in trauma ICU population over 5 days after ICU admission is around 18%. Therefore, to obtain 70 patients of AKI during 5 days of follow-up, we enrolled 418 trauma patients. However, to reduce the cost of NGAL kits, NGAL test was not done in all the patients. The samples were refrigerated and NGAL tests were done only in 70 patients of AKI and 70 patients of non-AKI (selected by computer generated randomization).

Plasma and urine NGAL tests were performed in 70 healthy volunteers from blood donors in our institutional blood bank to know the baseline value of NGAL in normal population.

The clinical team used the RIFLE criteria as reference gold standard to define AKI, which entails urine output and serum creatinine/glomerular filtration rate (GFR) criteria. Accordingly, the patient's hourly urine output and daily serum creatinine, and eGFR (estimated GFR) by modification of diet in renal disease (MDRD) equation were monitored. Patients were hence broadly classified as AKI or non-AKI. AKI severity was further staged into risk, injury, and failure as per RIFLE criteria[17,18] [Table 1].

At the time of ICU admission and subsequently at 24 and 48 h, 2 mL blood sample was collected in ethylenediaminetetraacetic acid anti-coagulated vacuette and 2 ml urine sample was collected and centrifuged at 800 × *g* for 5 min and then frozen at −80°C till further analysis. Serum creatinine was measured by fully automated CX-9 machine (Beckman coulter) for 5 days after admission. Six hourly urine output was also measured for 5 days. The samples were assayed for NGAL using R&D Systems human liopcalin-2/NGAL Quantikine Enzyme-linked immune sorbent assay kit. The NGAL assessment results were blinded to the treating medical team during the study period and there was no impact in the medical management of subjects. Daily measurement of eGFR by MDRD formula was calculated for 5 days. Injury severity score (ISS) was measured on admission. Sequential organ failure assessment (SOFA) and acute physiology, age, chronic health evaluation (APACHE) II were also recorded for 3 consecutive days following ICU admission. In-hospital mortality and length of stay were also observed.

Means and standard deviations were used to describe continuous variables. For skewed data, median and interquartile range was used. Categorical variables were expressed as proportions. Receiver operator characteristic (ROC) analysis was used to assess the ability of NGAL to predict AKI within 48 h when measured on admission. ROC curves were presented and the area under the curve (AUC) was calculated. Sensitivity, specificity, positive and negative likelihood ratios, and positive and negative predictive values were reported for a wide range of thresholds. The ability of NGAL measured at 24 h and 48 h to predict subsequent AKI was explored to evaluate the benefit of obtaining serial NGAL measurements over time. Spearman's rank correlation was calculated to find the correlation between NGAL (plasma and urine) and outcome (ICU and hospital length of stay as well as in-hospital mortality).

## Results

Study flow chart of participants is depicted in Figure 1. Out of 986 patients admitted in the ICU during the study period (August 2014 to January 2017), 418 patients fulfilled the inclusion criteria and were followed-up for 5 consecutive days. The reasons of exclusion in 568 patients as per the exclusion criteria are mentioned in Figure 1. As per the RIFLE criteria (serum creatinine/urine output volume), 70 patients were broadly classified as AKI and rest of the patients (n = 348) were classified as non-AKI. Out of 348 non–AKI patients, only 70 patients were further analyzed for pNGAL and uNGAL on admission, at 24 and 48 h. These 70 non-AKI patients were selected out of 348 patients by computer-generated randomization. Patient demographics, that is, age, weight, and gender were comparable in both the groups (AKI vs non-AKI). As per RIFLE criteria, 56 (80%) and 14 (20%) patients developed AKI, 24 and 48 h after injury, respectively. The risk, injury, and failure category comprised 34 (48.58%), 18 (25.71%), and 18 (25.71%) AKI patients, respectively, while none of the patients were observed in loss and end stage category [Table 2]. The median ICU and hospital length of stay were significantly longer in non-AKI patients. Mortality was significantly higher in AKI patients as compared to non-AKI patients [Table 3]. Comparison of various characteristics (ISS, SOFA, and APACHE II scores, serum creatinine, 6-h urine output, and e GFR) in AKI and non-AKI group are presented in Table 4. The mean pNGAL value on ICU admission, at 24 h and at 48 h was significantly higher in AKI patients compared with the non-AKI patients. The mean uNGAL was also significantly elevated at 48 h [Table 5].

Serial measurement (at the time of admission, at 24 h, and 48 h) of pNGAL and uNGAL were done to determine

**Table 1: RIFLE classification for AKI**

| RIFLE Classification | Creatinine and GFR Criteria | Urine output Criteria |
|---|---|---|
| Risk | Increased S. creat × 1.5 or GFR decrease >25% | <0.5 ml/kg/h ×6 h |
| Injury | Increased S. creat × 2 or GFR decrease >50% | <0.5 ml/kg/h × 12 h |
| Failure | Increased S. creat × 3 or GFR decrease >75% or S. creat ≥4 mg/dl | <0.3 ml/kg/h × 24 h or anuria × 12 h |
| Loss | Persistent acute renal failure=complete loss of kidney function >4 weeks | |
| End stage kidney disease | End stage kidney disease (need for dialysis >3 months) | |

*RIFLE=risk, injury, failure, loss, and end stage kidney disease. AKI=acute kidney injury, S. creat=serum creatinine. GFR=glomerular filtration rate*

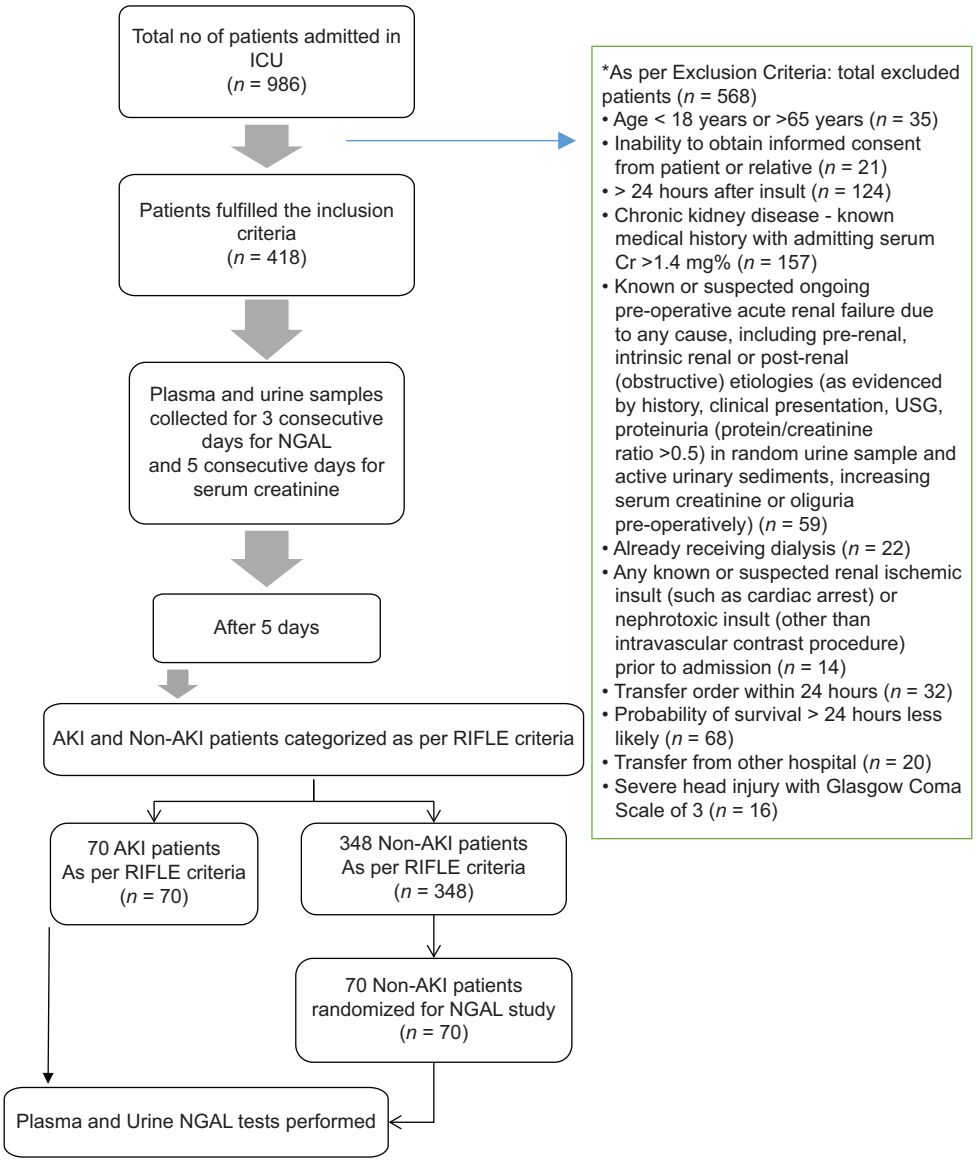


**Figure 1:** Study flow chart of participants

their predictive ability for development of AKI within 120 h of admission in patients to the ICU. The area under the receiver operative characteristic curve (AUROC) on admission was 0.84 and at the cut-off point of 125.40 ng/mL(95% CI: 0.78 to 0.91), had a sensitivity of 75.7%, and a specificity of 74.29% [Figure 2a]. The 24-h pNGAL value with the cut-off point was 122.38 ng/mL (95% CI: 0.82491 to 0.93570), had a sensitivity of 82.86%, specificity of 78.57%, and AUROC was 0.88 [Figure 2b]. Similarly, the 48-h pNGAL with the cut-off point of 122.20 ng/mL (95% CI: 0.80798 to 0.92713), had sensitivity and specificity of 78.57% and 77.14%, respectively; and the AUROC was 0.87 [Figure 2c]. The uNGAL measured on admission, at the cut-off of 16.23 ng/mL (95% CI: 0.48480 to 0.67561), to predict the development of AKI within 120 h of ICU admission had a sensitivity and specificity of 61.43% and 60%, respectively (AUROC 0.58) [Figure 3a]. The uNGAL measured at 24 h with the cut-off of 16.06 ng/mL (95% CI: 0.51457 to 0.70298) had a sensitivity and specificity of 61.43% and 61.43%, respectively (AUROC 0.61) [Figure 3b]. Similarly, the uNGAL measured at 48 h with the cut-off of 16.24 ng/mL (95% CI: 0.62553 to 0.79856) had a sensitivity of 70% and specificity of 65.71% (AUROC 0.71) [Figure 3c]. The AUROC, cut-off values, sensitivity, and specificity of pNGAL and uNGAL are given in Table 6. The cut-off value of median pNGAL and uNGAL on admission, at 24 h and 48 h for the risk, injury, and failure category as per RIFLE criteria is given in Table 7.

The baseline value of mean pNGAL in normal healthy population was 11.709 ± 2.372 ng/mL (Median: 12.320 [2.291 to 14.441 ng/mL]). Mean uNGAL level

in healthy population was 3.084 ± 1.619 ng/mL (Median: 3.399 [−0.906 to 5.835 ng/mL]) [Table 8]. There was no statistically significant association of pNGAL and uNGAL (measured on admission, at 24 and 48 h) with ICU length of stay, however, both pNGAL and uNGAL had negative correlation with the ICU length of stay. pNGAL and uNGAL measured at 48 h were significantly associated with hospital length of stay ($P$ = 0.04 and 0.02, respectively) [Table 9]. Increased mean pNGAL at all time points (on admission, at 24 h, and 48 h) was significantly associated with mortality ($P$ = 0.01), while higher mean uNGAL on admission and at 48 h was significantly associated with mortality ($P$ = 0.01) [Table 10].

**Table 2: Demographics, time period between injury to development of AKI, severity of AKI as per RIFLE criteria**

| Parameters | AKI ($n$=70) | NonAKI ($n$=70) | $P$ |
|---|---|---|---|
| Age (years) [mean±SD] | 37.06±12.56 | 36.34±13.54 | 0.75 |
| Weight (Kg) [mean±SD] | 69.29±8.67 | 67.04±8.10 | 0.12 |
| Gender [%] | | | |
| Female | 10 (55.56) | 8 (44.44) | 0.61 |
| Male | 60 (49.18) | 62 (50.82) | |
| Time period between injury to development of AKI (hours) [%] | | | |
| 24 h | 56 (80) | NA | - |
| >48 h | 12 (20) | | |
| Severity of AKI as per RIFLE classification [$n$ (%)] | | | |
| Risk | 34 (48.58) | NA | - |
| Injury | 18 (25.71) | | |
| Failure | 18 (25.71) | | |
| Loss | 0 (0) | | |
| End stage | 0 (0) | | |

*AKI=acute kidney injury, RIFLE=risk, injury, failure, loss, and end stage kidney disease. ICU=intensive care unit, LOS=length of stay*

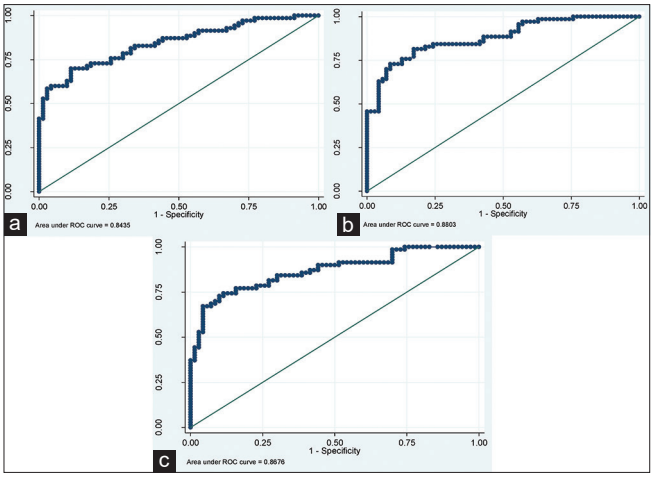


**Figure 2:** Area under receiver operator characteristic curves demonstrating the predictive ability of plasma NGAL on admission (a), at 24 h (b), and 48 h (c) to predict AKI upto 48 h. AKI = acute kidney injury, AUROC = area under the receiver operator characteristic curve, NGAL = neutrophil gelatinase-associated lipocalin

## Discussion

AKI is a common complication of severe trauma. Incidence of AKI in trauma patients in surgical ICU is high (30% to 50%), and severity of AKI is independently associated with mortality.[19] Early diagnosis of AKI and identification of the underlying etiology are essential to guide timely interventions. NGAL has been used for the prediction of AKI among critically ill patients but there is paucity of clinical studies esablishing its role as a diagnostic marker for adult trauma patients admitted in ICU, without any prior kidney disease/ renal complications. The cause of AKI in the ICU patients could be due to preexisting comorbidities.[20] In this study, we tried to overcome these problems by excluding patients with any preexisting renal disorder/injury and by studying the sequential predictive value of both plasma and urinary NGAL at different time points from ICU admission.

We observed that 80% patients developed AKI after 24 h of ICU admission while 20% patients developed AKI after 48 h of ICU admission, with a maximum RIFLE category risk, injury, and failure in 48.58%, 25.71%, and 25.71%, respectively. Our study has shown the predictive value of pNGAL on admission with AUROC 0.84 [Figure 2a], at 24 h with AUROC 0.88 [Figure 2b], and at 48 h with AUROC 0.87 [Figure 2c], while predictive value of uNGAL on admission, at 24 h, and at 48 h were AUROC 0.58, 0.61, and 0.71, respectively [Figure 3a-c]. The AUROC in our study is highly consistent with that reported in 19 studies comprising 2538 patients across different AKI settings in a meta-analysis by Haase *et al.*[21] This meta-analysis comprised all the studies which included plasma and/or urine NGAL as a diagnostic marker. The cut-off value of NGAL (both pNGAL and uNGAL) concentration for optimal sensitivity

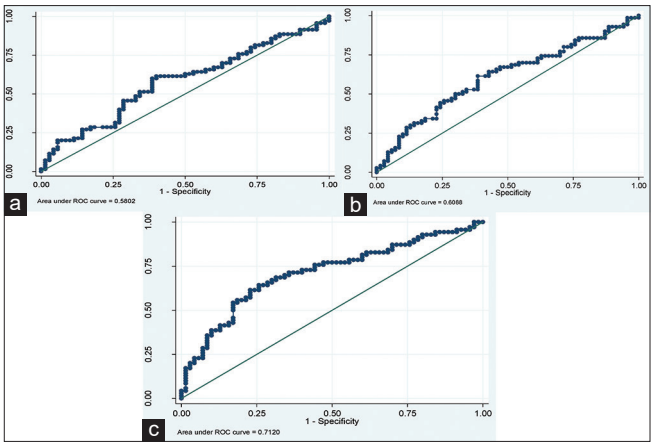


**Figure 3:** Area under receiver operator characteristic curves demonstrating the predictive ability of urine NGAL on admission (a), at 24 h (b), and 48 h (c) to predict AKI upto 48 h. AKI = acute kidney injury, AUROC = area under the receiver operator characteristic curve, NGAL = neutrophil gelatinase-associated lipocalin

**Table 3: Secondary outcome measures- ICU length of stay, hospital length of stay and mortality in AKI and non-AKI patients**

| Parameters | AKI (*n*=70) | Non-AKI (*n*=70) | *P* |
|---|---|---|---|
| ICU LOS (days) [median (range)] | 7 (2-32) | 11.5 (3-79) | 0.01* |
| Hospital LOS (days) [median (range)] | 10 (3-87) | 17 (5-180) | 0.01* |
| ICU LOS (days) in alive patients [median (range)] | 9 (2-32) | 10 (3-140) | 0.36 |
| Hospital LOS (days) in alive patients [median (range)] | 16 (5-87) | 17 (5-180) | 0.84 |
| In hospital mortality [%] | | | |
| Yes | 39 (55.71) | 9 (12.86) | 0.01* |
| No | 31 (44.29) | 61 (87.14) | [difference 42.9 (95% CI 28.9, 56.9) *P* value 0.01] |

*AKI=acute kidney injury, ICU=intensive care unit, LOS=length of stay, CI=confidence interval. * Significant P value*

**Table 4: Comparative data of ISS, SOFA, and APACHE II scores, serum creatinine, urine output, and eGFR in AKI, and non-AKI group**

| Parameters | Time (hours) | AKI (*n*=70) | Non-AKI (*n*=70) | *P* |
|---|---|---|---|---|
| ISS | 0 (on admission to ICU) | 16 (4-34) | 9 (4-25) | 0.01* |
| SOFA [median (range)] | 0 | 5.5 (1-13) | 5 (2-12) | 0.79 |
| | 24 | 7 (1-15) | 5 (2-11) | 0.01* |
| | 48 | 7 (2-16) | 4.5 (1-14) | 0.01* |
| APACHE II [mean±SD] | 0 | 17 (5-28) | 15 (6-24) | 0.01* |
| | 24 | 17 (5-29) | 14 (5-25) | 0.01* |
| | 48 | 16.5 (5-28) | 14 (5-24) | 0.01* |
| S. creatinine (mg%) [median (range)] | 0 | 0.65 (0.2-1.3) | 0.8 (0.4-1.3) | 0.02* |
| | 24 | 1 (0.4-4.8) | 0.7 (0.3-0.1.2) | 0.01* |
| | 48 | 1 (0.4-5.6) | 0.6 (0.3-1.2) | 0.01* |
| | 72 | 0.9 (0.3-6.7) | 0.6 (0.3-1.09) | 0.01* |
| | 96 | 0.9 (0.4-6.2) | 0.6 (0.3-1.1) | 0.01* |
| Urine output (mL/6 hourly) [median (range)] | 0 | 300 (200-580) | 565 (300-895) | 0.01* |
| | 24 | 250 (30-600) | 600 (350-750) | 0.01* |
| | 48 | 245 (20-600) | 600 (350-850) | 0.01* |
| | 72 | 250 (10-580) | 600 (400-800) | 0.01* |
| | 96 | 250 (10-550) | 615 (450-850) | 0.01* |
| eGFR (mL/min/1.73 $m^2$) [median (range)] | 0 | 139 (56-507) | 114.82 (56-262) | 0.03* |
| | 24 | 84.54 (14-210.44) | 130.31 (63-365) | 0.01* |
| | 48 | 85.94 (11-272.25) | 143.06 (49.57-415.04) | 0.01* |
| | 72 | 91.5 (9-246) | 148.27 (61.18-341) | 0.01* |
| | 96 | 96.50 (9-285.91) | 151 (54.81-336) | 0.01* |

*AKI=acute kidney injury, ICU=intensive care unit, S. creatinine=serum creatinine. ISS=injury severity score, APACHE II=acute physiology, age, chronic health evaluation II. SOFA=sequential organ failure assessment, eGFR=estimated glomerular filtration rate. * Significant P value*

**Table 5: Plasma and urinary NGAL measurement on three consecutive days (on admission, 24 hours and 48 hours after admission) in AKI and non-AKI group**

| Parameters | Time (hours) | AKI (*n*=70) | Non-AKI (*n*=70) | *P* |
|---|---|---|---|---|
| pNGAL (ng/mL) [mean±SD] | 0 | 204.08±91.25 | 93.74±45.36 | 0.01* |
| | 24 | 216.73±89.14 | 94.63±48.16 | 0.01* |
| | 48 | 212.77±97.12 | 86.32±49.11 | 0.01* |
| uNGAL (ng/mL) [mean±SD] | 0 | 14.16±4.89 | 13.50±4.76 | 0.42 |
| | 24 | 14.69±4.14 | 13.85±4.22 | 0.23 |
| | 48 | 15.45±3.57 | 13.48±4.45 | 0.01* |

*AKI=acute kidney injury, pNGAL=plasma NGAL, uNGAL=urine NGAL. NGAL=neutrophil gelatinase-associated lipocalin. * Significant P value*

and specificity to predict AKI across all settings ranged from 100 to 270 ng/mL, while in our study, the cut-off of pNGAL and uNGAL ranged from 122.20 to 125.40 ng/mL and 16.06 to 16.23 ng/mL, respectively. Similar results were observed in a prospective study by Fodor *et al.*,[22] wherein authors observed that pNGAL had a better discrimination

**Table 6: Cut-off value, sensitivity, and specificity of plasma and urine NGAL measured at admission, at 24 h, and 48 h to predict AKI within 96 h of ICU admission**

| Parameters | $pNGAL_0$ | $pNGAL_{24}$ | $pNGAL_{48}$ | $uNGAL_0$ | $uNGAL_{24}$ | $uNGAL_{48}$ |
|---|---|---|---|---|---|---|
| AUROC | 0.84 | 0.88 | 0.87 | 0.58 | 0.61 | 0.71 |
| Cut-off (ng/mL) | 125.40 | 122.38 | 122.20 | 16.23 | 16.06 | 16.24 |
| Sensitivity | 75.7% | 82.86% | 78.57% | 61.43% | 61.43% | 70% |
| Specificity | 74.29% | 78.57% | 77.14% | 60.00% | 61.43% | 65.71% |

*NGAL=neutrophil gelatinase-associated lipocalin. AKI=acute kidney injury, $pNGAL_0$ : plasma NGAL on admission to ICU, $pNGAL_{24}$ : plasma NGAL at 24 hours and $pNGAL_{48}$ : plasma NGAL at 48 hours, $uNGAL_0$ : urine NGAL on admission to ICU, $uNGAL_{24}$ : urine NGAL at 24 hours and $uNGAL_{48}$ : urine NGAL at 48 hours. AUROC=area under the receiver operating characteristic curve. ICU: Intensive care unit*

**Table 7: Cut-off values of plasma and urine NGAL for various stages of AKI**

| Parameters | RIFLE (On the basis of Serum Creatinine) (*n*=70) | | |
|---|---|---|---|
| | Risk (*n*=34) | Injury (*n*=18) | Failure (*n*=18) |
| pNGAL (ng/mL) [median (range)] | | | |
| On admission | 170.65 (33.06-333.03) | 238.39 (64.01-342.45) | 232.90 (90.63-342.79) |
| At 24 h | 179.93 (77.75-343.19) | 238.38 (60.03-344.90) | 264.58 (122.38-351.08) |
| At 48 h | 158.36 (56.00-346.72) | 268.70 (90.69-350.46) | 259.60 (93.70-353.36) |
| uNGAL (ng/mL) [median (range)] | | | |
| On admission | 12.48 (0.51-18.72) | 15.60 (1.63-19.64) | 15.89 (11.60-18.23) |
| At 24 h | 14.66 (4.16-17.95) | 15.06 (5.47-18.41) | 14.39 (0.44-18.12) |
| At 48 h | 14.93 (2.95-18.13) | 16.51 (10.99-17.37) | 15.39 (2.28-19.20) |

*AKI=acute kidney injury, pNGAL=plasma NGAL, uNGAL=urine NGAL, RIFLE=risk, injury, failure, loss, and end stage kidney disease. NGAL=neutrophil gelatinase-associated lipocalin*

**Table 8: Cut-off value of plasma and urine NGAL in healthy volunteers (*n*=70)**

| Parameters | Mean | SD | Median | Min | Max |
|---|---|---|---|---|---|
| pNGAL (ng/mL) | 11.709 | ±2.372 | 12.320 | 2.291 | 14.441 |
| uNGAL (ng/mL) | 3.084 | ±1.619 | 3.399 | −0.906 | 5.835 |

*pNGAL=plasma NGAL, uNGAL=urine NGAL, NGAL=neutrophil gelatinase-associated lipocalin*

**Table 9: Correlation of plasma and urine NGAL with the ICU and hospital length of stay**

| Parameters | ICU length of stay (days) | | Hospital length of stay (days) | |
|---|---|---|---|---|
| | *r* | *P* | *r* | *P* |
| pNGAL (ng/mL) | | | | |
| On admission | −0.06 | 0.48 | −0.08 | 0.32 |
| At 24 hours | −0.11 | 0.21 | −0.14 | 0.09 |
| At 48 hours | −0.07 | 0.43 | −0.17 | 0.04* |
| uNGAL (ng/mL) | | | | |
| On admission | −0.09 | 0.25 | −0.10 | 0.22 |
| At 24 hours | 0.10 | 0.22 | 0.00 | 0.99 |
| At 48 hours | −0.09 | 0.30 | −0.20 | 0.02* |

*Data represented in P value and correlation coefficient (r). pNGAL=plasma NGAL, uNGAL=urine NGAL, ICU=intensive care unit. NGAL=neutrophil gelatinase-associated lipocalin. * Significant P value*

capability for early prediction of AKI (AUROC = 0.81) in critically ill patients compared with previously used biomarkers. They suggested that the use of this novel biomarker may allow early protective renal intervention for critically ill patients.

The timing and the predictive capability of pNGAL and uNGAL vary in previous studies. Cruz *et al.*[23] found that plasma NGAL is a useful early diagnostic marker for AKI development within 48 h (AUROC 0.78, 95% CI [0.65–0.90]); Using a cut-off value of 150 ng/ml for pNGAL, the sensitivity and specificity was 73% and 81%, respectively; however, its performance was low to predict AKI when extended to 5 days (AUROC 0.67, 95% CI [0.55–0.79]) with a sensitivity and specificity of 46% and 80%, respectively. They also observed that peak plasma NGAL concentrations increased with worsening AKI ($R = 0.55$, $P = 0.01$), and hence pNGAL can be used as a predictor for RRT use during the ICU stay (AUROC 0.82, 95% CI 0.70–0.95). Our study results show that both pNGAL and uNGAL can be valuable diagnostic and predictive biomarker of AKI. De Geus *et al.*[24] conducted a prospective cohort study in 632 adult ICU patients. The authors reported AUROCs ranged from 0.77 (RIFLE R) to 0.86 (RIFLE F) for pNGAL and from 0.80 (RIFLE R) to 0.88 (RIFLE F) for uNGAL. This study concluded that development of severe AKI can be predicted by the measurement of NGAL at ICU admission including in patients with normal serum creatinine. Matsa *et al.*[25] found that on admission, both pNGAL and uNGAL can predict the development of AKI upto 72 h after ICU admission (AUROC 0.766 and 0.791, respectively). We reported early predictive ability of pNGAL at admission but contrary to Matsa study results, our study shows that uNGAL lag behind pNGAL in diagnosis of early AKI on admission. In the study by Matsa, there was sequential decrease in the number of patients in whom the serial NGAL measurement was done with time (0, 24, and 48 h). The number of patients

**Table 10: Association of plasma and urine NGAL with mortality**

| Parameters | In hospital mortality | | |
|---|---|---|---|
| | Yes ($n$=48) | No ($n$=92) | *P* |
| | Mean±SD | | |
| pNGAL (ng/mL) [mean±SD] | | | |
| On admission | 200.87±101.22 | 121.8±71.39 | 0.01* |
| At 24 hours | 213.51±96.34 | 125.51±77.71 | 0.01* |
| At 48 hours | 217.65±109.41 | 114.01±72.29 | 0.01* |
| uNGAL (ng/mL)[mean±SD] | | | |
| On admission | 15.54±3.34 | 12.94±5.23 | 0.01* |
| At 24 hours | 14.91±3.64 | 13.94±4.43 | 0.19 |
| At 48 hours | 15.64±3.12 | 13.85±4.47 | 0.01* |

*Data represented in P value and mean, SD. pNGAL=plasma NGAL, uNGAL=urine NGAL. NGAL=neutrophil gelatinase-associated lipocalin. *Significant P value*

were not equal at different time period, while in our study, we assessed equal number of patients at all time points.

Siew *et al.*[26] suggested that on admission, uNGAL values independently associated with detection of subsequent AKI within a heterogeneous ICU population (AUROC 0.71). However, as a stand-alone marker using modest creatinine-based cut-offs, uNGAL seems to have limited utility beyond a conventional clinical risk-prediction model. Similar to our study, Mahmoodpoor *et al.*[27] found that pNGAL (AUROC 0.76) on admission was more sensitive marker than uNGAL (AUROC 0.53) to predict progressive AKI in the ICU. They also showed that both plasma and urine levels of NGAL failed to predict early and late mortality of patients who were admitted to the ICU with AKI. Apart from ICU patients, NGAL has also been observed to be a predictor of AKI in patients undergoing cardiac surgery,[28] patients with preexisting CKD,[29] acute pancreatitis,[30] post liver transplant,[31] or after severe infections.[32] The predictive ability of pNGAL versus uNGAL in all above condition are variable with few studies demonstrating that uNGAL is a better predictor than pNGAL. The cut-off value of pNGAL and uNGAL in normal healthy Indian population has not been studied so far. Hence, we included 70 healthy donors. The cut-off value of pNGAL and uNGAL were 11.71 ± 2.37 ng/mL and 3.08 ± 1.62 ng/mL, respectively. Similar pNGAL (14.59 ± 3.71 ng/mL) and uNGAL (5.5 ± 6.3 ng/mL) values were observed by Gharishvandi *et al.*[33] and Krzeminska *et al.*[34] in healthy controls.

We observed significant association between mortality and NGAL; pNGAL was significantly associated with mortality at all time point, while uNGAL was significantly associated with mortality on admission and at 48 h. Interestingly, the length of stay in ICU and hospital was more in the non-AKI group as compared with AKI group of patients. Both pNGAL and uNGAL had negative correlation with the ICU and hospital length of stay. This is due to higher mortality in the AKI group as compared with non-AKI group. The performance of NGAL for predicting AKI in more heterogeneous ICU populations has been inconsistent (AUROCs ranging from 0.54 to 0.92).[35] NGAL is produced in the kidney in response to tubular injury and is mainly secreted into urine. These findings provide biological plausibility for uNGAL as an AKI biomarker.[11] However, despite ongoing large-scale research, absolute proof of NGAL's role in predicting AKI is still lacking.

Our study has certain strength and limitations. The strength of this study is the relatively large critically ill trauma patients, and inclusion of both plasma as well as urine NGAL. We also included the normal healthy volunteers to establish the cut-off value of pNGAL and uNGAL in Indian subpopulation. Limitation of our study includes the fact that it was a single center study with heterogeneous trauma ICU patients. Secondly, we included only adult trauma patients. We also excluded all patients with prerenal disorder, cardiac arrest, and patients with GCS score of 3. Another limitation is that urine output was not measured every hour, but 6 hourly measurement was being done and average hourly urine output was calculated. To limit the expenditure, NGAL tests were done in only 70 patients out of the 348 non-AKI patients. This may have led to exclusion of few non-AKI patients as per RIFLE criteria but with high NGAL value, although this limitation was negated by selecting 70 non-AKI patients for NGAL test by computer-generated randomization. There was significant difference between the AKI and non-AKI group in terms of ISS, SOFA, and APACHE II scores. This could also be a confounding factor in analyzing the data and weakness of the control group.

In conclusion, our study showed that in trauma ICU patients, pNGAL measured at admission can predict AKI occurrence up to 96 h post-ICU admissions with a sensitivity of 75.7% and specificity of 74.29% and the sensitivity and specificity of uNGAL to detect AKI is 61.43% and 60.00%, respectively. The accuracy of uNGAL improved slightly as patients progresses through their ICU stay. Serial measurement of pNGAL and uNGAL showed that they can be useful as an early and potent biomarker to predict AKI and in-hospital mortality. The cut-off value of pNGAL and uNGAL in healthy population was 11.709 ng/mL and 3.084 ng/mL, respectively. We conclude that pNGAL had good predictive value on admission (AUROC 0.84), while uNGAL had moderate performance (AUROC 0.58). pNGAL can be used as an early and potent diagnostic and predictive marker of AKI and mortality in critically ill trauma patients.

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## Conflicts of interest

There are no conflicts of interest.

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