Introduction

Acute kidney injury (AKI) is a presentation of an underlying heterogeneous group of conditions that leads to impairment of filtration and excretion of nitrogenous waste products from the body. A prompt early diagnosis to detect AKI is a mandate due to the associated risk of high mortality and morbidity. We tested the sensitivity and specificity of plasma neutrophil gelatinase-associated lipocalin (NGAL), a potential biomarker of AKI, versus serum creatinine, the gold standard laboratory test.

Materials and Methods: A cross-sectional diagnostic type study was conducted from February 2015 to January 2017 after obtaining the institutional ethics clearance certificate. Individuals admitted to the intensive care unit (ICU) of a tertiary care hospital of northeast India who were diagnosed with septicemia, heart failure, and ketoacidosis and individuals on nephrotoxic drugs such as aminoglycosides were included in the study. Serum creatinine and plasma NGAL of all individuals were estimated using suitable methods within 24 h of admissions. Results: Considering all inclusion and exclusion criteria, 138 individuals were included in the study. The area under the curve (AUC) for plasma NGAL on day 1 of admission was 0.800 (95% confidence interval [CI]: 0.712–0.882). In the study, we estimated a plasma NGAL cut-off value of 391 ng/mL (with an odds ratio of 9.89) within the day of admission.

Conclusion: Plasma NGAL is a candidate biomarker of AKI with acceptable sensitivity and specificity (AUC of 0.80) that can predict AKI in our setup before serum creatinine is raised, thereby asking for a prompt intervention to reduce the mortality and morbidity associated with AKI.

Keywords: AKI, creatinine, NGAL.

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waste products in the body that were previously filtered and excreted by the kidneys. AKI is designated for an underlying heterogeneous group of conditions that shares common diagnostic features.\textsuperscript{11} AKI is defined by KDIGO (Kidney Disease Improving Global Outcomes) guidelines either by a rise in serum creatinine by 0.3 mg in the last 48 h or an increase in serum creatinine by more than 1.5 times from the baseline value within 7 days or urine output lower than 0.5 mL/kg/h for 6 h.\textsuperscript{6} Globally, the incidence of AKI varies from country to country and there is an existence of regional variations. In a meta-analysis conducted by Susantitaphong et al.\textsuperscript{19} that included 154 different studies from all over the world found an incidence of AKI varying from 24.9\% (confidence interval [CI]: 22.1–27.8) in the United States to 15.6\% (CI: 8.7–26.4) in Asia. A higher incidence of AKI has been reported from critically ill patients in intensive care units (ICUs) with a noteworthy point that there are incidences of AKI in rural settings in regions that are situated in the south of the equator. The cause of incidence in the rural setting is due to gastroenteritis and other infections such as malaria, leptospirosis, and hemolytic uremic syndrome.\textsuperscript{10} A hospital-based study conducted by Korula et al.\textsuperscript{10} from Kochi, Kerala, India, reported the incidence of AKI to be 16.1\% among hospitalized critically ill patients. Limited data exist on the incidence of AKI from the northeastern part of India.\textsuperscript{10} The risk of AKI is multifactorial ranging from septicemia, nephrotoxins, trauma, and heart failure with comorbidities such as diabetes mellitus and neoplasia. Mohammed et al.\textsuperscript{10} reported sepsis (49.2\%) as the leading cause of hospital-acquired AKI in their study from the ICU of a tertiary care hospital in India.

AKI is a life-threatening condition with increased mortality and significant morbidity with the development of end-stage renal disease.\textsuperscript{18} There is also evidence of a higher risk to develop AKI in pregnant women due to preeclampsia or heart failure.\textsuperscript{16} The risk of development of AKI increases with age and critically ill patients but using the available criteria for risk stratification, the prediction of AKI declines with increasing age.\textsuperscript{11} Thus, early diagnosis of at-risk individuals is the key to preventing the development of AKI as well as reducing the mortality and morbidity associated with AKI.\textsuperscript{18} Until now, as per the definition of AKI, creatinine is the gold standard laboratory test to detect AKI.\textsuperscript{5} Creatinine is synthesized in the muscles from creatinine phosphate and hence is dependent on muscle mass.\textsuperscript{18} Serum creatinine takes 24 to 36 h to rise following a renal injury.\textsuperscript{14} There is a requirement of baseline reference range, which is not applicable in the case of individuals with CKD as they often have a raised serum creatinine value.\textsuperscript{13} As prompt diagnosis is required for a better outcome of AKI, many predictive models have been designed using different serum and urine biomarkers. Angiotensinogen is one of them, others include neutrophil gelatinase-associated lipocalin (NGAL), Kidney injury molecule-1 (KIM-1), Inteleukin 18 (IL18), Liver fatty acid binding protein (L-FABP) with different sensitivities and specificities.\textsuperscript{14} NGAL is produced from distal nephrons during the early post-ischemic kidney injury. NGAL is an ion transporter whose synthesis is upregulated in AKI.\textsuperscript{17} Hypoxia induces the synthesis of NGAL in individuals with aortic valve diseases and this increases the synthesis of NGAL, which may be to favor the extracellular remodeling of the damaged tissue.\textsuperscript{18} Mishra et al.\textsuperscript{19} reported a study on 71 children who underwent coronary artery bypass graft (CABG) and were evaluated for serum and urine NGAL by enzyme-linked immunosorbent assay (ELISA) and western blotting. For 20 children who developed AKI following bypass surgery, serum and urine NGAL increased significantly from the baseline within 2 h in contrast to serum creatinine, which was increased from day 1 to 3. The study conducted by Soyler et al.\textsuperscript{20} reported an area under the curve (AUC) of 0.78 (95\% CI: 0.66–0.90) with respect to urine NGAL, indicating the highest positive predictive value of urine NGAL for prompt diagnosis of AKI at a cut-off value of 12 ng/mL in the first 72 h.

Because AKI is an acute condition, it needs immediate intervention to reverse the ischemic insult due to it. So far, serum creatinine was used as a biomarker for AKI whose expression usually occurs after 24 to 36 h of ischemic injury, necessitating the physicians to depend on other biomarkers to detect AKI early. Keeping the above-mentioned points in mind, plasma NGAL was used as a testing tool to compare with serum creatinine, which is the gold standard laboratory test to diagnose AKI.

Objectives

- To compare the sensitivity and specificity of plasma NGAL with serum creatinine.
- To estimate the cut-off of plasma NGAL for AKI in our setup.

Materials and Methods

Study design: Cross-sectional diagnostic type study

Inclusion criteria: Individuals admitted to the ICU of a tertiary care hospital of northeast India who were diagnosed with septicemia, heart failure, and ketoacidosis and individuals on nephrotoxic drugs such as aminoglycosides. Individuals with trauma who were hemodynamically stable and postoperative cases were also included in the study.

Exclusion criteria: Individuals on renal replacement therapy, those with chronic kidney disease, traumatic crush injury, and individuals with an abnormal hemogram.

The study was approved by the institutional ethics committee. Informed consent of all individuals was taken; where it was not possible, consent was taken from the first-degree relatives after verifying from the registration card.

Study period: February 2015 to January 2017.

Sample: As the samples of the admitted individuals were sent to the Biochemistry Laboratory every 24 h for vital biochemical parameters such as random blood sugar, blood urea, serum creatinine, and serum electrolytes, 2 mL of

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ethylenediaminetetraacetic acid (EDTA) sample was also sent for the estimation of plasma NGAL. The patient's samples were followed up for 7 days, wherever it was possible.

Analysis: Serum creatinine was estimated using the Beckman Coulter AU 480 Biochemistry Random-access Analyzer (Beckman Coulter, USA) using the Jaffe kinetic initial rate assay. The assay was calibrated using calibrators supplied by Beckman Coulter. Stringent third-party quality control was maintained by Lyphochek chemistry control (BioRad, USA).

Plasma NGAL was estimated by the particle enhanced turbidometric immunoassay method using reagents supplied by Biopporto diagnostics (Denmark). The method was programmed and calibrated in the Beckman Coulter AU 480 biochemistry random access analyzer using multilevel calibrators supplied by Biopporto with the concentration of the highest calibrator up to 3000 ng/mL.

Statistics: All data were entered into a Microsoft Office Excel sheet and analyzed by SPSS version 22 (IBM, Chicago, USA).

### Results

Considering all inclusion and exclusion criteria, 138 individuals were included in the study. To test the homogeneity of the plasma NGAL and serum creatinine, an analysis of variance (ANOVA) test was done [Table 1] that showed a highly significant result. Table 2 shows the correlation coefficients of Spearman correlation between plasma NGAL and serum creatinine with a correlation coefficient of 0.668. The result was statistically highly significant ($P = 0.000$) with a two-tailed distribution. The relationship of serum creatinine and plasma NGAL was in line with our study with a coefficient of 0.611 [Table 3]. Figures 1 and 2 show the receiver operating characteristics (ROC) curve of plasma NGAL for detecting AKI. The AUC for day 1 of admission was 0.800 (95% CI: 0.712–0.882) [Figure 1]. The AUC with respect to the plasma NGAL is shown in Figure 2, with a higher AUC of 0.864 (95% CI: 0.772–0.956). The AUC of plasma NGAL in the following days of admission is listed in Table 4. From the table, it is evident that the AUC of different days of admissions had an increasing trend, the highest being 96.2% on day 5 of admission to ICU.

### Discussion

To become a biomarker of AKI, a candidate should have the property of specificity to tubular injury so that it is expressed from the site of injury and should be detectable in the population at risk. NGAL is a biomarker that has been frequently and extensively studied in various literature across the world. In our study, it was noted that the serum creatinine and plasma NGAL concentration were non-homogeneous in nature, indicating that the concentrations varied from day to day with an increasing trend with the development of AKI [Table 1]. In a study that included 231 admitted patients, out of which 78 had developed the hospital-acquired AKI, the concentration of plasma NGAL was significantly higher in individuals who developed AKI with a mean of 295 ± 228 vs. 129 ± 108 ng/mL in those who did not have AKI. Mishra et al also reported a significantly higher NGAL concentration from baseline in pediatric individuals who developed AKI following coronary artery bypass graft (CABG). As several studies have until now reported that plasma NGAL rises well ahead between

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**Table 1: Results of ANOVA to test the homogeneity of plasma NGAL**

| Model   | Sum of squares | df  | Mean square | $F$   | Sig. |
|---------|----------------|-----|-------------|-------|------|
| Regression | 95542802.593 | 1   | 95542802.593 | 181.643 | 0.000|
| Residual  | 160427553.245 | 305 | 525991.329 | 0.000 |      |
| Total    | 255970157.838 | 306 |              |       |      |

*Dependent variable: NGAL. Predictors: (constant), creatinine*

**Table 2: Correlation is significant at the 0.01 level (two-tailed) during the study conducted from February 2015 to January 2017**

| Parameter | Creatinine | NGAL |
|-----------|------------|------|
| Correlation coefficient | 1.000 | 0.668** |
| Sig. (two-tailed) | 0.000 | 0.000 |
| n | 307 | 307 |

**Spearman's correlation**

**Table 3: Linear regression model between serum creatinine and plasma NGAL**

| Model | Unstandardized coefficients | Standardized coefficients | t   | Sig. |
|-------|-----------------------------|---------------------------|-----|------|
|       | B | Std. error | Beta | | |
| (Constant) | 461.415 | 53.100 | 8.690 | 0.000 |
| Serum creatinine | 185.249 | 13.745 | 0.611 | 13.478 | 0.000 |

*Dependent variable: NGAL*

**Table 4: Area under the curve for plasma NGAL for various days with reference to serum creatinine**

| Days of admission | AUC | Std. error | 95% Confidence interval | Lower bound | Upper bound |
|-------------------|-----|------------|-------------------------|-------------|-------------|
| Day 3             | 0.944 | 0.030 | 0.885 | 1.000 |
| Day 4             | 0.937 | 0.046 | 0.847 | 1.000 |
| Day 5             | 0.962 | 0.044 | 0.875 | 1.000 |
| Day 6             | 0.889 | 0.105 | 0.684 | 1.000 |
| Day 7             | 0.917 | 0.095 | 0.731 | 1.000 |
24 and 48 h before the rise in serum creatinine, an attempt was made in our study to correlate plasma NGAL with serum creatinine using Spearman correlation [Table 2]. In our study, the plasma NGAL concentration was well correlated with serum creatinine with a correlation coefficient of 0.668. This correlation was statistically significant at a P value of 0.01 (two-tailed distribution). Many studies have reported a strong direct correlation of plasma NGAL with serum creatinine. A modest correlation of \( r = 0.42 \) was reported by Maisel et al., who studied 29 individuals who developed AKI with a significance of \( P < 0.001 \). Bachorzewska-Gajewska et al. reported a correlation coefficient of variation (\( r^2 \)) = 0.65 at 2 h with serum creatinine following coronary angiography. In a study that included 118 AKI individuals with hypertensive disorders of pregnancy, a correlation coefficient of 0.4 was found. These findings are almost similar to our findings.

To test the association between serum creatinine and NGAL, we used the linear regression model [Table 3]. The dependent variable plasma NGAL was significantly related to serum creatinine with a coefficient of 0.611. Many studies have reported the association of urine NGAL with AKI and urine NGAL was found to be a good predictor of the development of AKI with an AUC of 0.78 (95% CI: 0.66–0.90) in the study that enrolled 100 heart failure patients. The study conducted by Chen et al. on 149 individuals who developed AKI, reported a binary logistic regression model for AKI prediction using heart failure, CKD, and serum bilirubin. They reported an AUC of 0.81 with 69.8% sensitivity with a specificity of 83.4%. Vanmassenhove et al. reported a strong correlation between serum NGAL and urine NGAL in both individuals who developed AKI and those who did not. The AKI group had an \( r^2 \) value of 0.38 versus 0.31 in the non-AKI group in a model of linear regression.

NGAL is one of the potential biomarkers of AKI that is detectable in the plasma 2 h following ischemia produced by CABC due to increased hepatic synthesis. It is filtered by the glomerulus and secreted by the proximal tubule. Urine NGAL is detectable in 3 h with a peak at 6 h. Sustained elevation of up to 5 days has been reported by some literature. As both plasma and urine NGAL exert molecular and cellular events of AKI, both are good candidates to predict the onset and course of AKI. No statistical differences in discriminating AKI were noted when plasma NGAL was combined with soluble urokinase plasminogen activator receptor (suPAR) than plasma NGAL alone by Walls in his study (AUC of combined plasma NGAL and suPAR of 0.82 vs. AUC of 0.78 of plasma NGAL). This indicates that plasma NGAL itself has reasonably acceptable sensitivity and specificity. Serum NGAL was also found to be elevated very early in AKI due to COVID-19 and urinary NGAL was reported to be associated with the severity of outcome due to AKI in COVID-19 patients.

An AUC of 0.87 had been reported earlier by Aqeel et al. who studied the AUC of many other biomarkers along with it. In our study, we found an AUC of 0.80 (95% CI = 0.718–0.882) [Figure 1]. Because we estimated the plasma NGAL daily

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**Table 1:**

| Area   | Std. error | 95% confidence interval |
|--------|------------|-------------------------|
| 0.800  | 0.042      | 0.718–0.882             |

**Figure 1:** ROC curve showing the sensitivity and specificity of plasma NGAL with reference to serum creatinine in day 1 of admission

**Figure 2:** ROC curve showing the sensitivity and specificity of plasma NGAL with reference to serum creatinine on day 2 of admission

| Area   | Std. error | 95% confidence interval |
|--------|------------|-------------------------|
| 0.864  | 0.047      | 0.772–0.956             |
for up to 7 days, the AUC of NGAL on day 2 was 0.864 (95% CI = 0.772–0.956). The AUC of the following days was listed in Table 4. In a noteworthy study, Kashani et al.\(^1\) studied many biomarkers such as urine and plasma NGAL, plasma cystatin-C, KIM-1, IL-18, pi-GST, and urine L-FABP by enrolling 744 individuals from two centers; they reported an AUC of approximately 0.7 of plasma NGAL with higher AUC in the case of urine NGAL. However, plasma NGAL AUC was reported to be 0.91 and that of urine NGAL was 0.998 at 2 h in the GALLANT trial following cardiac surgery.\(^2\) These differences in the AUC may be due to different methods that are being used to measure NGAL\(^3\) and we assume are partly.

Due to these existing differences, it will be beneficial if we set our cut-off to predict AKI in our setting, which is also one of the objectives of our study. Table 4 shows the AUC for plasma NGAL on different days up to 7 days of admission to ICU. Because plasma NGAL is raised very early at 2 h following an ischemic insult to the kidneys,\(^4\) we will consider the NGAL cut-off value of 391 ng/mL (with an odds ratio of 9.89) within the day of admission. Kashani et al.\(^5\) had reported a cut-off value of 323 ng/mL for AKI progression, which was associated with an odds ratio of 7.7 following cardiac surgery. Wheeler et al.\(^6\) in their study found a median serum NGAL value of 302 ng/mL (Interquartile range: 151–570 ng/mL). A similar finding was also found in a study conducted by Prabhu et al.\(^7\) who estimated 4-h post-CABG plasma NGAL of 352.97 ± 49.32 who developed AKI vs. 199.83 ± 23.28 ng/mL, \(P = 0.000\) who did not.

**Limitations**

Despite our best efforts with the available resources, considering the high cost of these new biomarkers as compared to the cost of creatinine, our study had many limitations. We have included only hospital-acquired AKI. There is a possibility that the cohort of community-acquired AKI could have been a separate characteristic. Due to different methods of estimation of NGAL and creatinine and different population subsets, the cut-off value of our setup may not work universally. Thus, a scientific meta-analysis or a multicenter trial may be required involving a large population.

**Conclusion**

We would like to conclude that plasma NGAL is a candidate biomarker of AKI with acceptable sensitivity and specificity (AUC of 0.80). With a cut-off value of 391 ng/mL, plasma NGAL can predict AKI in our setup before a rise in serum creatinine in the blood. This can be utilized by the physicians for AKI risk stratification to reduce mortality and morbidity.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Waikar SS, Bonventre JV. Acute kidney injury. In: Kasper DL, Fauci AS, Hauser SL, Longo, DL, Larry JJ, Loscaizo J, editors. Harrison's Principles of Internal Medicine. 20th ed. New York, NY: Mc Graw Hill Education; Chapter 279. p. 2293-308.

2. Foreword. Kidney Int Suppl (2011) 2012;2:2338.

3. Susantitaphong P, Cruz DN, Cerda J, Abuflaraj M, Alqahtani F, Koulouridis I, et al. Acute kidney injury advisory group of the American society of nephrology. World incidence of AKI: A meta-analysis. Clin J Am Soc Nephrol 2013;8:1482-93.

4. Cerda J, Bagga A, Kher V, Chakravarthi RM. The contrasting characteristics of acute kidney injury in developed and developing countries. Nat Clin Pract Nephrol 2008;4:138-53.

5. Korula S, Balakrishnan S, Sundar S, Paul V, Balagopal A. Acute kidney injury-incipience, prognostic factors, and outcome of patients in an Intensive Care Unit in a tertiary center: A prospective observational study. Indian J Crit Care Med 2016;20:332-6.

6. Bhattacharya PK, Roy A, Jamil M, Barman B, Murti SV, Marak PR. Clinical profile and determinants of short-term outcome of acute kidney injury: A hospital-based prospective study from Northeastern India. J Lab Physicians 2019;11:5-10.

7. Mohammed ZA, Suresh AA, Kumar P, Atturet RP. Acute kidney injury: Prevalence and outcomes in southern Indian population. J Clin Diag Res 2018;12:LC05-10.

8. Sileanu FE, Murugan R, Lucko N, Clermont G, Kane-Gill SL, Handler SM, et al. AKI in low-risk versus high-risk patients in intensive care. Clin J Am Soc Nephrol 2015;10:187-96.

9. Hoste EA, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: The international AKI-EPI study. Intensive Care Med 2015;41:1411-23.

10. Cooke WR, Hemmilä UK, Craik AL, Mandula CJ, Mvula P, Msusa A, et al. Incidence, aetiology and outcomes of obstetric-related acute kidney injury in Malawi: A prospective observational study. BMC Nephrol 2018;19:25.

11. Kane-Gill SL, Sileanu FE, Murugan R, Trietley GS, Handler SM, Kellum JA. Risk factors for acute kidney injury in older adults with critical illness: A retrospective cohort study. Am J Kidney Dis 2015;65:860-9.

12. Malhotra R, Kashani KB, Macedo E, Kim J, Bouchard J, Wynn S, et al. A risk prediction score for acute kidney injury in the intensive care unit. Nephrol Dial Transplant 2017;32:814-22.

13. Rodwell VW. Conversion of Amino Acids to Specialized Products. Harper's Illustrated Biochemistry. 30th ed. New York, NY: McGraw-Hill Education. Chapter 30. 2015. p. 313-22.

14. Ostermann M, Joannidis M. Acute kidney injury 2016: Diagnosis and diagnostic workup. Crit Care 2016;20:299.

15. Ostermann M. Diagnosis of acute kidney injury: Kidney disease improving global outcomes criteria and beyond. Curr Opin Crit Care 2014;20:581-7.

16. Alge JL, Arthur JM. Biomarkers of AKI: A review of mechanistic relevance and potential therapeutic implications. Clin J Am Soc Nephrol 2015;10:147-55.

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17. Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL): A new marker of kidney disease. Scand J Clin Lab Invest Suppl 2008;241:89-94.

18. Swaminathan G, Krishnamurthy VK, Sridhar S, Robson DC, Ning Y, Grande-Allen KJ. Hypoxia stimulates synthesis of neutrophil gelatinase-associated lipocalin in aortic valve disease. Front Cardiovasc Med 2019;6:156.

19. Mishra J, Dent C, Tarabishi R, Mitsuves MM, Ma Q, Kelly C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2003;365:1231-8.

20. Soyler C, Tanriover MD, Ascioglu S, Aksu NM, Arici M. Urine neutrophil gelatinase-associated lipocalin levels predict acute kidney injury in acute decompensated heart failure patients. Ren Fail 2015;37:772-6.

21. Schrenzenmeier EV, Barasch J, Budde K, Westhoff T, Schmidt-Ott KM. Biomarkers in acute kidney injury-Pathophysiological basis and clinical performance. Acta Physiol (Oxf) 2017;219:554-72.

22. Kashani K, Cheungpasitporn W, Ronco C. Biomarkers of acute kidney injury: The pathway from discovery to clinical adoption. Clin Chem Lab Med 2017;55:1074-89.

23. Palazzuoli A, Ruocco G, Pellegrini M, De Gori C, Del Castillo G, Franci B, et al. Comparison of neutrophil gelatinase-associated lipocalin versus B-Type natriuretic peptide and cystatin C to predict early acute kidney injury and outcome in patients with acute heart failure. Am J Cardiol 2015;116:104-11.

24. Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, et al. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care 2013;17:R25.

25. Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med 2008;36:1297-303.

26. Prabhhu A, Sujatha DI, Ninan B, Vijayalakshmi MA. Neutrophil gelatinase associated lipocalin as a biomarker for acute kidney injury in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass. Ann Vasc Surg 2010;24:525-31.