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Research Article

Serum Neutrophil gelatinase-associated lipocalin, a novel biomarker for prediction of AKI development in critically ill patients

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Abstract

Background: Acute kidney injury (AKI) is a frequent complication in critically ill patients and is associated with high morbidity and mortality; therefore, its prophylaxis, diagnosis and intervention positively impact patient evolution. Neutrophil gelatinase-associated lipocalin (NGAL) is thought to be a novel biomarker of AKI of several etiologies and is increased in both serum and urine before the increase of serum creatinine. **Method:** A prospective cohort study was conducted on 50 critically ill patients in ICU. Patients were stratified into 2 groups based on AKI development. Group I which included 25 patients who developed AKI, and group II which included 25 patients who did not develop AKI. AKI was defined based on acute kidney injury network (AKIN) classification. The Sequential Organ Failure Assessment (SOFA) scores were also calculated for all patients. Detailed medical history, demographic data and routine laboratory investigations were done. Serum NGAL was measured upon admission to ICU and upon AKI development. **Results:** 29 males and 21 females were included in the study with mean age 37.64years in group I, and 39.56 years in group II. In group I, as regards risks of AKI development, 8% of patients had developed shock, 24% were dehydrated, 12% had contrast exposure, 12% had rhabdomyolysis, 16% developed bleeding, and 28% had history of NSAID intake recently. On admission, there were no significant differences between the patients in both groups as regards Serum creatinine, hemoglobin level and other laboratory parameters ($p > 0.05$). Serum levels of NGAL were significantly higher in group I, before AKI development, with mean value 1081.72ng/ml, when compared with group II with mean value 211.65ng/ml ($p<0.01$). Serum NGAL levels were also significantly higher in group I after AKI development when compared with the levels before AKI development in the same group (1878.10ng/ml) ($p<0.01$). There was no significant correlation between NGAL levels and AKIN classification stages. **Conclusion:** In the absence of diagnostic increases in serum creatinine, NGAL detects patients with likely subclinical AKI who have an increased risk of adverse outcomes. Reassessment of AKI classification and stages should be considered.

Keywords: NGAL, AKI, sepsis, ICU.

Introduction

The incidence of AKI has been rising in recent year with negative influence on overall outcomes (1). Community-acquired AKI is common at admission to the emergency department (ED) (2), and the diagnosis still largely relies on serial serum creatinine (SCr) measurements, a delayed and nonspecific marker (3).Acute kidney injury (AKI) is frequent in critically ill patients admitted to intensive care units (ICUs) and is independently associated with increased morbidity and mortality (4).NGAL, also known as lipocalin-2 (lcn2), is a 25-kDaprotein and member of the lipocalin super family (5). It was named after its expression in neutrophils and found to have bacteriostatic effects by interfering with bacterial siderophore-mediated iron uptake (6). NGAL expression has been shown to increase in response to inflammationin epithelial cells regularly exposed to microorganisms and in response to cellular oxidative stress (4). Increases in plasma NGAL have been reported in a wide range of systemic diseases, including acute infections, pancreatitis, heart failure, and cancer, but in recent years the potential role of plasma and urinary NGAL as early markers of AKI has been studied (4).

Materials and Methods

This prospective cohort study was conducted on 50 critically ill patients in ICU. Patients were stratified into 2 groups based on AKI development. Group I which included 25 patients who developed AKI, and group II which included 25 patients who did not develop AKI.AKI was defined as abrupt (within 48 hours) reduction in kidney function according to acute kidney injury network definition (AKIN) classification into:

- Stage 1: ≥ 0.3 mg/dL or 1.5 to 2 fold rise from baseline in createnine or urine output less than 0.5 mL/kg/hour for more than 6 hours.
- Stage 2: 2-3 folds rise from baseline of creatinine or urine output less than 0.5 mL/kg/ hour for more than 12 hours.
- Stage 3 :>3 folds rise from baseline of creatinine or acute rise of at least 0.5mg/dL or >4 mg/dL in serum creatinine level or urine output less than 0.3 mL/kg/ hour for 24 hours or anuria for 12 hours.

Detailed medical history, demographic data and routine laboratory investigations were done. Estimation of serum NGAL by enzyme- Linked immunosorbent assay (ELISA) was done twice, within 24 hour of admission and at time of AKI development (stage 1) only.

Statistical analysis

Data were collected, revised, coded and entered to the statistical package for social science (SPSS) version 17. The qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations and ranges. Comparison between two groups with qualitative data were done by using Chi square test and Fisher exact test was used instead of Chi square when the expected count in any cell found less than 5.Comparison between two groups with quantitative data were done by using Independent t-test when the distribution of the data found parametric and Mann-Whitney test was used with the non parametric data. Spearman correlation coefficients were used to assess the significant relation between two quantitative parameters in the same group. Receiver operating characteristic curve (ROC) was used to assess the best cut off point with sensitivity, specificity, positive predictive value (+PV) and negative predictive value (-PV).The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

P > 0.05: Non significant.
P < 0.05: Significant.
P < 0.01: Highly significant.

Results

Tables 1, 2 and 3 showed the demographic and laboratory parameters for both groups. Risks for AKI development in both groups are shown in table 4. No statistically significant difference between both groups as regards results of laboratory parameters at admission and as regards risks for AKI development. Serum levels of NGAL were significantly higher in group I, before AKI development, when compared with group II ($p<0.01$). Serum NGAL levels were also significantly higher in group I after AKI development when compared with the levels before AKI development in the same group ($p<0.01$) as shown in table 5. Serum NGAL is a good predictor of AKI development with a specificity96% and sensitivity 72% as shown in figure 1. In AKI group, no significant correlations between serum NGAL and laboratory parameters were found, except for hemoglobin level, as illustrated in table 6. Serum NGAL was also not correlated with AKIN classification stages as shown in table 7. Serum NGAL was good predictor for initiation of hemodialysis in AKI patients with specificity 71.4% and sensitivity 63.6% as shown in figure 2.NGAL was poorly correlated with patients outcome with specificity 68% and sensitivity 50% as shown in figure 3.

Table (1): Distribution of the studied group as regards age

	Groups				P Value
	Group I		Group II		
	Mean	± SD	Mean	± SD	
Age	37.64	13.81	39.56	13.26	> 0.05

Table (2): Distribution of the studied group as regard sex

		Groups				P Value
		Group I		Group II		
		Count	%	Count	%	
Sex	Female	10	40.0%	11	44.0%	0.774
	Male	15	60.0%	14	56.0%	
Total		25		25		

Table (3): Laboratory parameters of the studied groups

	Groups				P Value
	Group I		Group II		
	Mean	± SD	Mean	± SD	
Hgb (gm/dl)	10.62	3.09	10.78	2.1	> 0.05
WBCs X 10^3	8.07	2.41	8.2	1.7	> 0.05
Plts X 10^3	182.88	95.28	195.7	66.5	> 0.05
ALT U/L	47.24	31.77	50.2	22.5	> 0.05
AST U/L	54.96	40.22	61.5	54.3	> 0.05
Bilirubin Total mg/dl	1.15	.25	1.13	0.32	> 0.05
Bilirubin Direct mg/dl	.44	.20	0.52	0.35	> 0.05
INR	1.18	.17	1.05	0.15	> 0.05
Ca mg/dl	8.76	.77	9.2	0.45	> 0.05
PO_4mg/dl	4.13	.45	4.83	0.32	> 0.05
Albumingm/dl	3.60	.45	3.35	0.74	> 0.05
S. Creat on admission mg/dl	1.06	.24	1.15	0.53	> 0.05
BUN mg/dl	18.76	2.28	20.2	7.4	> 0.05
NaMmol/L	137.08	4.56	138.45	6.5	> 0.05
KMmol/L	3.95	.52	4.2	0.86	> 0.05

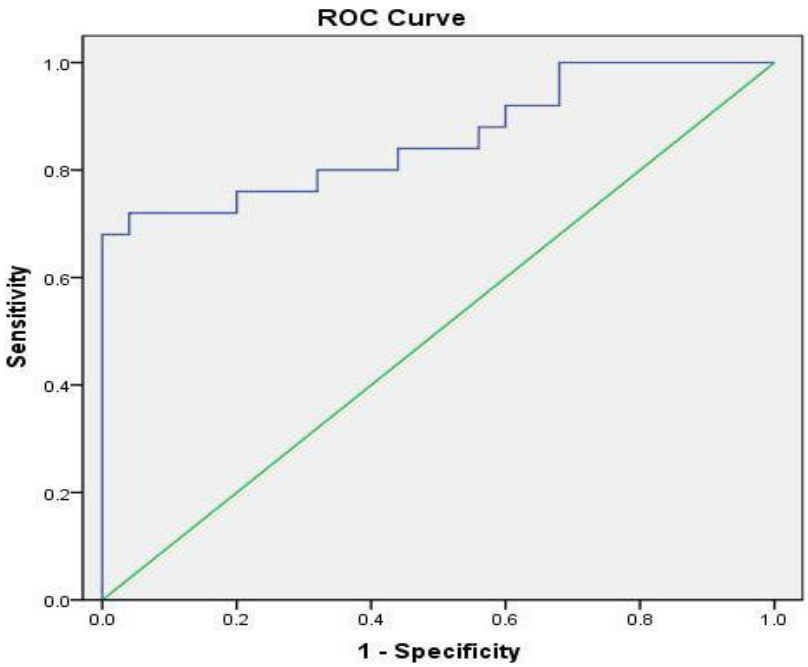
Table (4): Risks for AKI development.

		Groups				P Value
		Group I		Group II		
		Count	%	Count	%	
Risk of AKI	Shock	2	8.0%	3	12.0%	0.989
	NSAID	7	28.0%	7	28.0%	
	Dehydration	6	24.0%	5	20.0%	
	Contrast	3	12.0%	4	16.0%	
	Rhabdomyolysis	3	12.0%	3	12.0%	
	Bleeding	4	16.0%	3	12.0%	
Total		25		25		

Table (5): Serum NGAL levels in both groups.

	Groups				P Value
	Group I		Group II		
	Mean	± SD	Mean	± SD	
NGAL baseline ng/ml	1081.72	662.30	211.65	160.17	< 0.01
NGAL at AKI	1878.10	723.70			
P Value	< 0.01				

Figure (1): Serum NGAL as a predictor of AKI development



Cut Off	AUC 95% CI	Sensitivity	Specificity	PPV	NPV
≥ 600	85% (75.4 – 96.5)	72%	96%	94.7%	77.4%

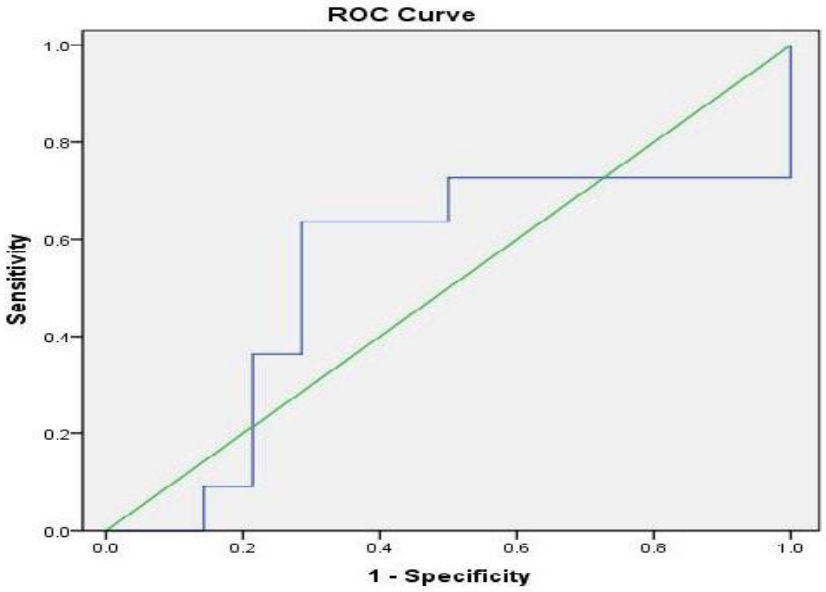
Table (6): NGAL Correlations with laboratory data in group I.

	NGAL baseline	
	Pearson Correlation	P Value
Age	-.082	.696
Hgbgm/dl	-.477*	.016
WBCs X10^3	.257	.216
Plts X10^3	.121	.565
ALT U/L	-.078	.710
AST U/L	-.115	.584
Bilirubin Total mg/dl	.157	.454
Bilirubin Direct mg/dl	.246	.236
INR	.190	.362
Ca mg/dl	-.078	.710
Po4 mg/dl	-.289	.161
Albumingm/dl	-.173	.410
Creat admission mg/dl	-.380	.061
BUN mg/dl	-.374	.066
NaMmol/L	-.280	.175
KMmol/L	-.110	.601
creat at AKI mg/dl	.101	.632
BUN at AKI mg/dl	-.257	.215
K at AKIMmol/L	.059	.779
urine volume	-.238	.251

Table (7): NGAL Correlations with AKIN classification stages

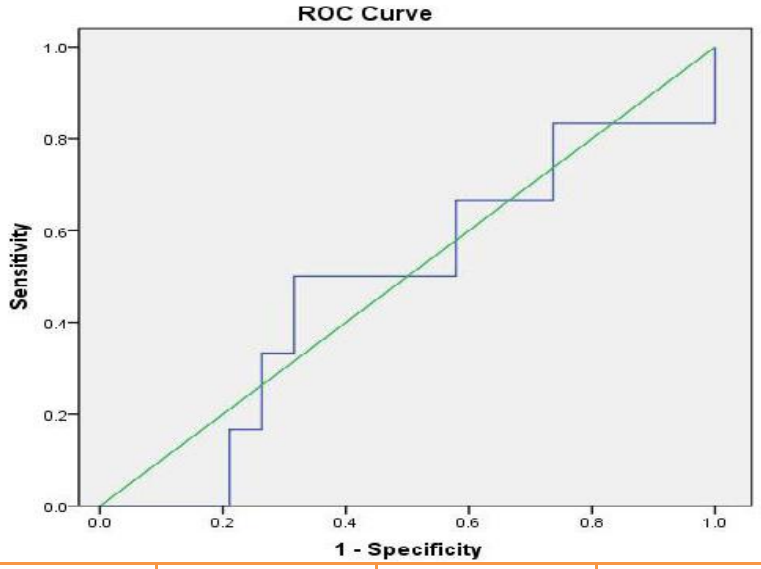
NGAL	AKIN_score						P Value
	Stage 1		Stage 2		Stage 3		
	Mean	± SD	Mean	± SD	Mean	± SD	
Baseline	1008.31	752.21	935.25	318.51	1236.58	630.92	0.576
AKI	1650.52	895.71	2198.61	228.52	2099.98	420.29	0.448

Figure (2): Serum NGAL as predictor for HD in AKI patients



Cut Off	AUC 95% CI	Sensitivity	Specificity	PPV	NPV
≥ 1268	53% (28.3 – 78.2)	63.6%	71.4%	63.6%	71.4%

Figure (3): NGAL as predictor of patients outcome.



Cut Off	AUC 95% CI	Sensitivity	Specificity	PPV	NPV
≥ 1418	48% (21.2 - 75.3)	50%	68%	33.3%	81.3%

Discussion

Acute kidney injury (AKI) is a common and serious condition, the diagnosis of which depends on serum creatinine measurements. Unfortunately, creatinine is a delayed and unreliable indicator of AKI. Fortunately, understanding the early stress response of the kidney to acute injuries has revealed a number of potential biomarkers. Neutrophil gelatinase-associated lipocalin is emerging as an excellent standalone troponin-like biomarker in the plasma and urine for the prediction of AKI, monitoring clinical trials in AKI and for the prognosis of AKI in several common clinical scenarios (7).The majority of studies on pNGAL has been performedin well controlled settings of hospital-acquired AKI, such as cardiac surgery, contrast administration, orcritically ill patients. NGAL measured at ICU admission predicts the development of severe AKI similarly to serum creatinine-derived eGFR. However, NGAL adds significant accuracy to this prediction in combination with eGFR alone or with other clinical parameters and has an interesting predictive value in patients with normal serum creatinine (8).The purpose of this study was to determine the accuracy of serum NGAL an early marker of AKI in critically ill patients. Data obtained from our study showed significant differences between levels of serum NGAL in patients who developed AKI and those who did not develop AKI. It showed that serum NGAL was good predictor for AKI development with a specificity 96% and sensitivity 72%. This was in agreement with many other clinical studies evaluating NGAL an early marker for prediction of AKI development. In a study conducted by Ramprasad Matsa et al., both urinary and serum NGAL were evaluated as predictors for the occurrence of AKI at an earlier point of time before the conventional markers changes (9). The results were in agreement with the results of our study. Also in a study conducted by AnjaHaase-Fielitzet al., NGAL was assessed as early predictor of AKI in three clinical settings, post cardiac surgery, critical illness and post kidney transplantation.. In all three settings, NGAL significantly improved the prediction of AKI risk over the clinical model alone (10). Our study showed that serum NGAL was a good predictor for hemodialysis initiation in AKI patients with specificity 71.4% and sensitivity 63.6%. This finding was in agreement with the finding obtained by Mahdavi-Mazdeh et al., in his study which revealed that serum NGAL level especially 24 hours post-transplantation, seems to be an early accurate predictor of both the need to dialysis and slow graft function within the first week of kidney transplantation (11).Other study conducted by Peter B Hjortrup et al., reported that NGAL seems to have reasonable value in predicting use of renal replacement therapy but not mortality (12). In our study, NGAL was poorly correlated with patients outcome with specificity 68% and sensitivity 50%.

Conclusion

Our study supported the use of NGAL as a biomarker for the prediction of AKI with high specificity. However, we noted some limitations, including lack of published studies that adhere to diagnostic study guidelines, heterogeneity in AKI definition, the lack of uniformly applicable cut-off values and variability in the performance of commercially available NGAL assays.

References

1-Karina Soto, Ana Luisa Papoila, Silvia Coelho, Michael Bennett, Qing Ma, Bruno Rodrigues, Pedro Fidalgo, Francisca Frade, and Prasad Devarajan: Plasma NGAL for the Diagnosis of AKI in Patients Admitted from the Emergency Department Setting. Clin J Am Soc Nephrol 8: 2053–2063, December, 2013.

2-Waikar SS, Liu KD, Chertow GM: Diagnosis, epidemiology and outcomes of acute kidney injury. Clin J Am Soc Nephrol 3: 844–861, 2008.

3-Pickering JW, Frampton CM, Endre ZH: Evaluation of trial outcomes in acute kidney injury by creatinine modeling. Clin J Am SocNephrol 4: 1705–1715, 2009.

4-Peter B Hjortrup, Nicolai Haase, Mik Wetterslev and Anders Perner: Clinical review: Predictive value of neutrophil gelatinase-associated lipocalin for acute kidney injury in intensive care patients. Critical Care, 17:211,2013.

5-Kjeldsen L, Cowland JB, Borregaard N: Human neutrophil gelatinase associated lipocalin and homologous proteins in rat and mouse. Biochim Biophys Acta, 1482:272-283, 2000.

6-Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK: The Neutrophil Lipocalin NGAL Is a Bacteriostatic Agent that Interferes with Siderophore-Mediated Iron Acquisition. Mol Cell, 10:1033-1043, 2002.

7-Prasad Devarajan: Neutrophil gelatinase-associated lipocalin: a promising biomarker for human acute kidney injury. Biomark Med,April ; 4(2): 265–280, 2010.

8-De Geus R, Bakker J and Lesaffre M: Neutrophil gelatinase-associated lipocalin at ICU admission

predicts for acute kidney injury in adult patients. Am J Respir Crit Care Med. April; 183(7):907-14, 2011.

9-RamprasadMatsa, Emma Ashley, Vivek Sharma, Andrew P Walden and Liza Keating: Plasma and urine neutrophil gelatinase-associated lipocalin in the diagnosis of new onset acute kidney injury in critically ill patients. Critical Care, 18:R137, 2014.

10-Anja Haase-Fielitz, Michael Haase and Prasad Devarajan: Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury a critical evaluation of current status. Ann Clin Biochem. May; 51(03): 335–351, 2014.

11-Mahdavi-Mazdeh M, Amerian M, Abdollahi A, Hatmi ZN, Khatami MR: Comparison of serum neutrophil gelatinase-associated lipocalin (NGAL) with serum creatinine in prediction of kidney recovery after renal transplantation. International journal of organ transplantation medicine.Volume 3. Issue 4,101:171, 2012.

12-Peter B Hjortrup, Nicolai Haase, MikWetterslev and Anders Perner: Predictive value of neutrophil gelatinase-associated lipocalin for acute kidney injury in intensive care patients. Critical Care, 17:211, 2013.