Original Article

Urine and serum neutrophil gelatinase-associated lipocalin cut-off point for the prediction of acute kidney injury

Alireza Merrikhi, Alale Gheissari, Hamideh Mousazadeh[1]

Department of Nephrology, Isfahan Kidney Diseases Research Center, Isfahan University of Medical Sciences, [1]Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: The aim of the present study was to determine the optimum cut-off point of urine and serum neutrophil gelatinase-associated lipocalin (NGAL) for the predictive diagnosis of acute kidney injury (AKI).

Materials and Methods: This study was a prospective observational study which was performed at Alzahra hospital and Emam Hussein Hospital, Isfahan, Iran. During a period of 4 months, from February 2012 to May 2012, consecutive patients admitted to pediatric intensive care unit (PICU) aged between 1 month and 15 years with glomerular filtration rate (GFR) more than 90 ml/min were enrolled in the study. In all the patients who were enrolled in the study, blood and urine samples were attained on the first, third, and fifth day of admission. Serum and urine NGAL were assessed and compared between patients who developed AKI and who didn't.

Results: Of 25 patients who enrolled in the study, 13 developed AKI. For the serum NGAL, the most accurate cut-off point was the fifth day cut-off point which was 163 375 pg/ml (sensitivity: 61.5%, specificity: 94.6%, AUC: 0.76) and urine NGAL cut-off point was 86 040 pg/ml (sensitivity: 50%, specificity: 92.5%, AUC: 0.73).

Conclusions: In conclusion, we deduced that serum NGAL level significantly elevates in critically ill patients admitted in PICU who develop AKI. Serum and urine NGAL on the fifth day are the best predictors for the AKI with cut-off points 163 375 and 86 040.

Key Words: Acute kidney injury, neutrophil gelatinase-associated lipocalin, sensitivity and specificity, RIFLE

Address for correspondence:
Dr. Hamideh Mousazadeh, Number 74, Banafsheh Alley, Azadegan Alley, Hezarjarib Street, Isfahan, Iran. E-mail: dr.mousazadeh@yahoo.com
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INTRODUCTION

Acute kidney injury (AKI) has been defined as the rapid loss of kidney function that results in the retention of urea and other nitrogenous waste products.[1-3] The loss of renal function is detected by measurement of the serum creatinine, but there are several limitations; first, serum creatinine level widely varies among individuals according to the muscle mass, age, and sex; second, serum creatinine level does not change until a considerable proportion of kidney function has been lost; third, renal function is overestimated at lower rates of glomerular filtration rate (GFR) due to rise in intubular secretion of creatinine.[4] Nowadays, serum creatinine is considered as a delayed and unreliable marker for the diagnosis of AKI, and to rely solely on this marker may lead to delay in the initiation of

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therapeutic interventions.[5,6]

Recent studies focused on finding new biomarkers for early detection of AKI. Several markers have been recognized such as interleukin-18 (IL-18),[7] kidney injury molecule-1 (KIM-1),[8,9] liver-type fatty acid-binding protein (L-FABP),[10] and neutrophil gelatinase-associated lipocalin (NGAL).

NGAL is known as well-established marker for early detection of AKI and several studies have been performed in this field.[11-13]

NGAL is a 25-kDa protein that bounds to matrix metalloproteinase-9 from neutrophils[14] which is expressed at a very low level in some tissues, and its level rises in injured epithelial cells, including the liver, lung kidney, and colon.[15] Previous studies mentioned that NGAL increases significantly in urine and serum compared with healthy controls and is correlated with serum creatinine. Its level rises 24 to 48 hours before creatinine level rises in AKI condition.[16] This marker helps the physician to early diagnose and manage AKI.

AKI occurs in approximately 80% of severely ill children in pediatric intensive care unit (PICU) which results in long hospital stay and increased patients' mortality rate.[17-19] Due to these rationales, AKI among these patients became one of the major concerns for physicians. As is it mentioned, it seems to be necessary to perform research projects in this field. In this study, we aimed to determine the optimum cut-off point of urine and serum NGAL for diagnosis of AKI.

MATERIALS AND METHODS

This study was a prospective observational study which was performed at Alzahra hospital and Emam Hossein Hospital, Isfahan, Iran.

Patients and setting

During a period of 4 months, from February 2012 to May 2012, consecutive patients admitted to pediatric intensive care unit (PICU) aged between 1 month and 15 years with GFR more than 90 ml/min were enrolled in the study. Patients were excluded if any of the following criteria were present: Patient dies before the end of study sampling, cannot obtain the blood sample, pre-existing renal insufficiency, and rejection of the patients' parents to participate in a research project.

Intervention and assessments

Of all patients, who were enrolled in the study, blood and urine samples were attained on the first, third, and fifth day of admission. Both samples were obtained by one of the research investigators (HM) at 20 O'clock on each day. Five milliliters of urine specimens were obtained from urine catheter. Three milliliters blood sample was withdrawn under aseptic conditions by suitable syringe according to patients' age and weight and immediately sent to hospital laboratory for further evaluations. Plasma NGAL level was assessed using human lipocalin-2/NGAL ELISA (Boster Biological Technology. Co., Ltd, USA).

Development of AKI was diagnosed using pediatric RIFLE classification, correspondence to the risk phase. Risk phase is defined as reduction in GFR $\geq$25% or creatinine rise $\geq$50% from baseline level, or a fall in urine output to <0.5 ml/kg/h for at least 8 hours. GFR were calculated using the Schwartz formula.[20]

The following data were also evaluated in all patients: Blood urea nitrogen (BUN), creatinine, urine output, urine creatinine. Severity of disease was assessed by the Acute Physiologic and Chronic Health Evaluation (APACHE) score[21] and the observational scale to identify serious illness in febrile children.[22] Further data including blood culture results, vasopressin, antibiotic and anti-seizure drug usage during ICU stay were also gathered.

Sample size were calculated using statistical formula considering $\alpha = 0.05$ and $\beta = 0.2$ to be 25 in this study. In this study, we also had 25 patients as the control group to evaluate the baseline NGAL in serum and urine. This control group consists of healthy children who came into Alzahra hospital with their parents for various reasons.

Statistical analysis

Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 16 software. Comparisons were made using Student *t*-test, ANOVA, Pearson regression, logistic regression, multiple regression analysis test as needed. Differences considered were significant if *P* value was <0.05. To calculate the diagnostic values for the plasma and urine NGAL levels at the cut-off points, receiver operating characteristic (ROC) curves were generated and the area under the curves (AUC) were calculated to determine the accuracy of NGAL as a biomarker.

Written informed consents were obtained from all patients and parents as needed, for authorize use of their medical records for research purposes with approval of the protocol by ethical committee of our university.

RESULTS

A total number of 25 patients including 18 (72%) males and 7 (28%) females were enrolled in the study. Of these patients who were admitted in ICU, 8 had

multiple trauma, 11 were with sepsis, and 6 were with cardiac diseases. 25 healthy children were also considered as the control group. Detailed data about age and sex of study participants and the control group are shown in Table 1.

Patients in the two study groups were compared for various characteristics and clinical outcomes including: Duration of ICU admission, deaths, APACHE score, observational scale, mechanical ventilation, and the administration of antibiotic, anti-seizure drugs, and vasopressin agents; however, none of them has statistically significant difference between two groups. Detailed data are shown in Table 2.

Data analysis for the correlation between serum and urine NGAL with APACHE score and observational scale was performed. Results showed that there is statistically significant correlation in the fifth day serum NGAL level with APACHE score and the observational scale; the others didn't have any significant correlation [Table 3 and Diagrams 1, 2].

We compared the values of urine and serum NGAL on the first, third, and fifth day of admission and baseline NGAL of the control group. We found a significant increase in serum NGAL on the third and fifth day compared with two other groups. Detailed data are shown in Table 4.

We analyzed data for the optimum cut-off point of serum NGAL for the prediction of AKI. Serum NGAL on the fifth day of admission was the most accurate for the prediction of AKI (AUC = 0.79); ROC curve of fifth day is presented in Figure 1.

The best cuts-off points for the first, third, and fifth day are 177755 pg/ml, 92720 pg/ml, and 163375 pg/ml, respectively, and the diagnostic values of these points

Table 1: Comparison of age, sex and BMI among study groups

	AKI (*N*=13)	No AKI (*N*=12)	Control (*N*=25)	
Male	9 (69.2%)	9 (75%)	16 (64%)	*P*=0.79
Age	12±11.8	83±51	17.3±17.2	*P*<0.001*
BMI	14.6±4.2	14.6±2.9	15.4±4.6	*P*=0.78

AKI: Acute kidney injury, BMI: Body mass index, Data are presented as number (%) and mean±standard deviation, *statistically significant difference

Table 2: Patients characteristic and clinical outcome

	AKI (*N*=13) (%)	No AKI (*N*=12) (%)	*P* value
Days of PICU stay	18.33±23.76	10.30±15.39	0.34
Observational scale	25.3±5.3	20.9±7.4	0.11
APACHE score	17.5±4.7	15.3±6.6	0.33
Days of mechanical ventilation	6 (46.15)	7 (58.33)	0.7
Deaths	5 (38.46)	4 (33.33)	0.99
Antibiotic usage	12 (92.30)	9 (75)	0.60
Anti seizure drug usage	9 (69.23)	7 (58.33)	0.69
Vasopressin agent usage	6 (46.15)	4 (33.33)	0.69

PICU: Pediatric intensive care unit, AKI: Acute kidney injury, APACHE: Acute physiologic and chronic health evaluation, Data are presented as number (%) and mean±standard deviation

Table 3: Serum and urine NGAL correlation with APACHE score and observational scale

	r	*P* value
APACHE score		
Serum NGAL		
First day	0.16	0.45
Third day	0.21	0.33
Fifth day	0.47	0.018*
Urine NGAL		
First day	0.21	0.3
Third day	0.35	0.09
Fifth day	0.28	0.18
Observational scale		
Serum NGAL		
First day	0.32	0.12
Third day	0.05	0.8
Fifth day	0.54	0.005*
Urine NGAL		
First day	0.31	0.13
Third day	0.34	0.1
Fifth day	0.32	0.12

APACHE: Acute Physiologic and chronic health evaluation, NGAL: Neutrophil gelatinase-associated lipocalin, *Statistically significant correlation

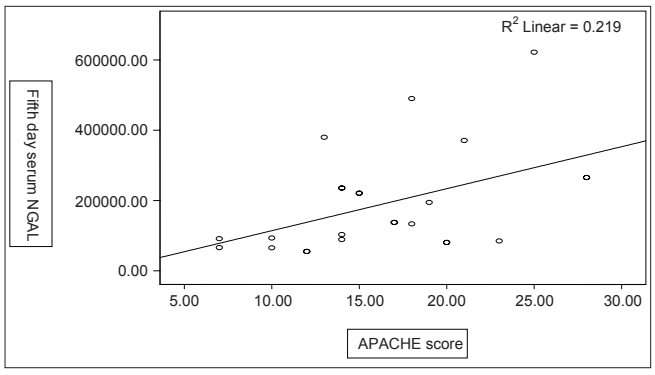

Diagram 1: Correletaion between APACHE score and the fifth day serum NGAL level

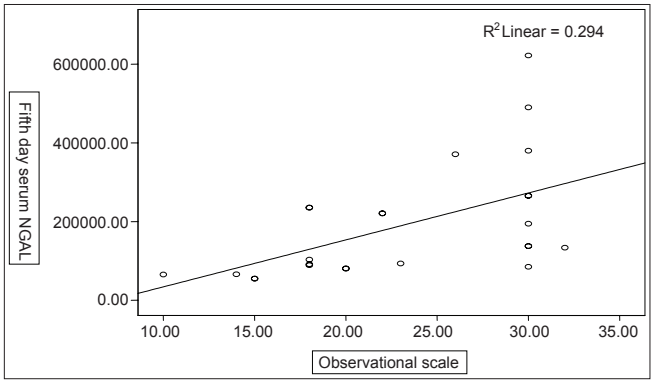

Diagram 2: Correletaion between observational scale and the fifth day serum NGAL level

and AUC are explained in Table 5.

Cut-off points for optimum accuracy were also determined for urine NGAL; fifth day urine NGAL is the most accurate for the prediction of AKI [Figure 2].

Optimum cut-off points for the first, third, and fifth day of admission were 65820 pg/ml, 66810 pg/ml, and 86040 pg/ml, respectively. Detailed data about the diagnostic value of NGAL for the prediction of AKI and AUC are shown in Table 6.

DISCUSSION

The aim of the present study was to determine the optimum cut-off point of urine and serum NGAL for the prediction of AKI. Our data showed that serum NGAL level significantly elevates in patients who develop AKI compared to those who do not. However, the increase in urine NGAL was not statistically significant. Although urine NGAL in AKI patients was at least 50-fold more than control value, the difference was not statistically significant in comparison to patients who didn't develop AKI. Serum and urine NGAL levels in the fifth day were the best predictors for the AKI with the cut-off values of 163 375 and 86 040.

The cut-off point for serum NGAL is within the range as the previous study.[23,24] Mc Donald *et al*. revealed the cut-off point of 89 000 pg/ml with the sensitivity of 68% and specificity of 70%, and AUC of 0.71.[23] In comparison to our data, they had relatively higher sensitivity and lower specificity and AUC values. The authors didn't report the PPV and NPV value at this cut off point. On the other hand, Fadel *et al*. suggested the value of 100 000 pg/ml as the cut-off point with the sensitivity and specificity of 100% and 90.5%, respectively.[24]

The urine NGAL in our study had the best optimum cut-off point of 86 040 pg/ml with the sensitivity of 46.1% and specificity of 91.6%. In a study conducted by Zapitelli *et al*. the highest and lowest cut-off points for sensitivity varied from 54% to 85% and for specificity varied from 97% to 44%. In their study, the AUC value for prediction of AKI was 0.79, which was higher than our study.[16] Another study performed by Wagener *et al*. suggested the cut-off point of 213 000 pg/ml for

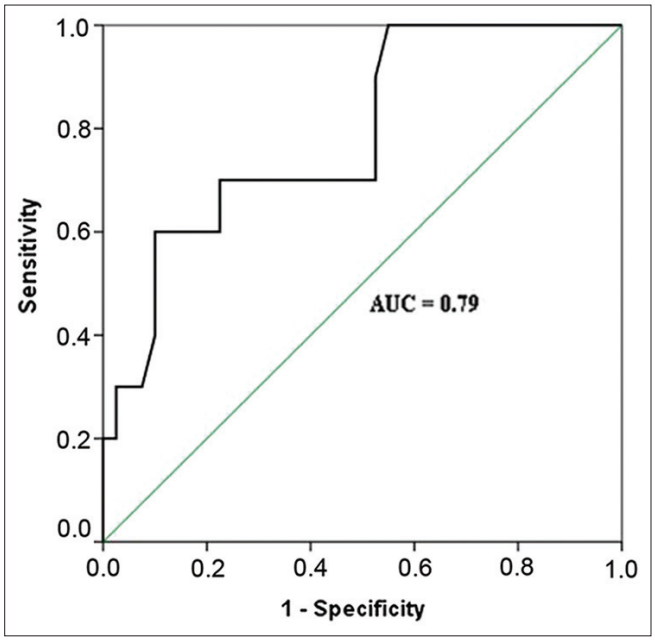

Figure 1: ROC curve for serum NGAL level on the fifth day of admission

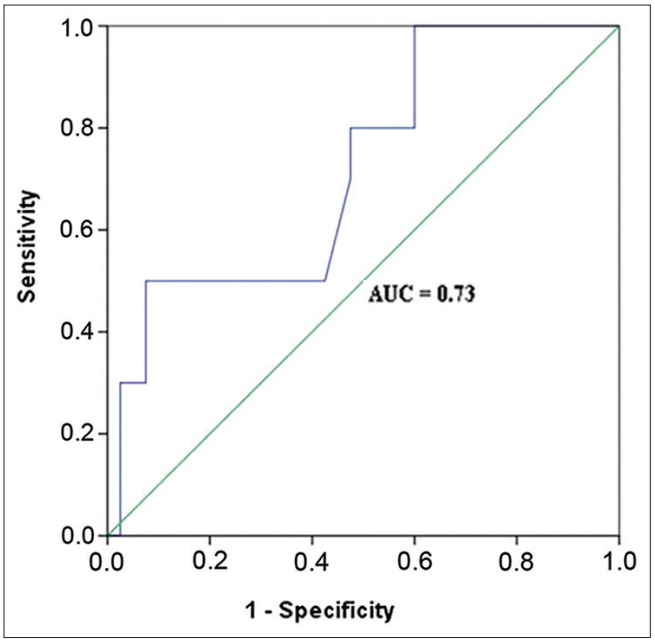

Figure 2: ROC curve for urine NGAL on the fifth day of admission

Table 4: The comparison of serum and urine NGAL among study patients and the control group

	Urine NGAL			Serum NGAL		
	First day	Third day	Fifth day	First day	Third day	Fifth day
AKI	11646±34077	5553±12409	6666±15981	247115±189389	370772±331320	235377±177664
No AKI	5444±9957	4559±7622	4038±6458	555085±127739	192542±16951	142924±84512
Control	110±132	-	-	137481±41382	-	-
P value	0.18	0.061	0.083	0.18	0.003*	0.021*

AK: Acute kidney injury, NGAL: Neutrophil gelatinase-associated lipocalin, Data are presented as mean±standard deviation, *statistically significant difference

Table 5: Diagnostic value of serum NGAL at the cutoff point values for the prediction of AKI

	First day AKI		Third day AKI		Fifth day AKI	
	Yes	No	Yes	No	Yes	No
Serum NGAL						
Positive	8	5	12	7	8	5
Negative	5	7	1	5	5	7
AUC	0.76		0.76		0.79	
Sensitivity (%)	61.5		92.3		61.5	
Specificity (%)	58.3		41.6		58.3	
PPV (%)	61.5		63.1		61.5	
NPV (%)	58.3		41.6		58.3	

AKI: Acute kidney injury; AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; NGAL: Neutrophil gelatinase-associated lipocalin

Table 6: Diagnostic value of urine NGAL at the cutoff point values for the prediction of AKI

	First day AKI		Third day AKI		Fifth day AKI	
	Yes	No	Yes	No	Yes	No
Urine NGAL						
Positive	8	2	7	1	6	1
Negative	5	10	6	11	7	11
AUC	0.72		0.72		0.73	
Sensitivity (%)	61.5		53.8		46.1	
Specificity (%)	83.3		91.6		91.6	
PPV (%)	80		87.5		85.7	
NPV (%)	66.6		64.7		61.1	

AKI: Acute kidney injury; AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; NGAL: Neutrophil gelatinase-associated lipocalin

urine NGAL with sensitivity and specificity of 73% and 78%, respectively.[25]

We also analyzed several parameters between two study groups. Data showed that the only parameter that had a significant difference between two groups was the patient's age which was significantly lower in patients who developed AKI. This point was not revealed in previous studies.[26]

Our study had some strength. First, all patients had normal kidney function at the beginning of the study, and the study design allowed us for the precise comparison with following changes. Second, in this study, we evaluated critically ill patients in PICU from various etiologies which differs our study from previous ones. We also had some limitations that were with our study design which was unblinded and observational. We suggest further multicenter studies with more powerful design for this purpose.

CONCLUSION

We deduced that serum NGAL level significantly elevates in patients who in critically ill patients who develop AKI when admitted in PICU. Serum and urine NGAL on the fifth day are the best predictors for the AKI with the cut-off points of 163 375 pg/ml and 86 040 pg/ml.

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