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Plasma and Urine Neutrophil Gelatinase-Associated Lipocalin (NGAL) as Early Predictor of Acute Kidney Injury in Septic Patients

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Abstract

Introduction: Sepsis is an important precipitant of acute kidney injury (AKI). Septic AKI may be characterized by a distinct pathophysiology. The lack of an early and effective biomarker results in a significant delay in initiating appropriate therapy. **Objective:** to determine the role of plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) as an early biomarker of acute kidney injury in septic patients. **Patients and methods:** Prospective, observational study enrolled 40 critically ill patients with sepsis and septic shock. Plasma and urine NGAL were measured at enrollment and 12 h. **Results:** Seventeen (42.5%) patients developed AKI. Patients with AKI, compared to those without, had significantly higher severity of illness, organ dysfunction, length of ICU stay, and more ICU mortality. AKI patients, compared to those without, had significantly higher baseline plasma NGAL (221.4 ± 104.7 vs. 129.5 ± 48 ng/ml, p = 0.003), and at 12 h (239.2 ± 122.9 vs. 128.9 ± 49.1 ng/ml, p = 0.002) and significantly higher urine NGAL at baseline (13.6 ± 5.5 vs. 7.8 ± 3 ng/ml, p = 0.001), and at 12 h (23.2 ± 6.5 vs. 15 ± 4.9 ng/ml, p = 0.0001). Baseline plasma NGAL ≥ 147 ng/ml had sensitivity of 82.4% and specificity of 73.9% for predicting AKI (AuROC 0.82), and urine NGAL at 12 h ≥ 19 ng/ml had sensitivity of 82.4% and specificity of 78.3% (AuROC 0.82). **Conclusion:** Plasma and urine NGAL are good predictors of AKI in critically ill septic patients

Keywords: Neutrophil gelatinase-associated lipocalin (NGAL) , Acute kidney injury (AKI) , Sepsis

1. Introduction:

Acute kidney injury (AKI) is a very common problem in critically ill patients. AKI incidence in adult intensive care unit (ICU) settings has been reported to range between 16% and 67% [1]. AKI in critically ill patients is associated with prolonged mechanical ventilation, a longer ICU stay, and increased rates of rehospitalization. Epidemiological studies have shown that half of the cases of AKI in ICU are related to sepsis, with a higher superimposed mortality than in non-septic AKI patients [2]. Sepsis is a significant cause of AKI [3]. Sepsis is responsible for 30-50% of all AKI encountered in critically ill patients [4]. Septic AKI carries a poorer prognosis with lower survival when compared with AKI of non-septic origin [5]. Septic AKI may be associated with higher rates of renal recovery [6]. Septic AKI is characterized by a distinct pathophysiology[7].These events may be reflected in unique patterns of plasma and urine biomarkers in septic AKI [8]. As a consequence, the application of traditional urinary biochemical and microscopy-based tests in the early diagnosis and differentiation of AKI may be misleading in septic AKI [8]. Early identification of primarily septic AKI may have clinical and prognostic importance. Serum creatinine concentrations and creatinine clearance are unreliable indicators of acute and abrupt changes in kidney function while patients are in the ICU [9]. Serum creatinine is a marker for worsening kidney function only after more than 50% of kidney function has been lost [10]. The lack of an early and effective biomarker results in a significant delay in initiating appropriate therapy [11]. Human studies have shown that AKI can be prevented and treated by several measures if they are instituted shortly after the initial insult to the kidney [2]. Neutrophil gelatinase-associated lipocalin (NGAL) is a rapidly emerging biomarker for early detection of acute AKI [12]. NGAL was identified as a 25-kDa protein bound to gelatinase from neutrophils [13]. Early recognition of AKI based on NGAL may facilitate earlier and more effective treatment than standard strategy [14].

2. Aim of the work:

To investigate the role of plasma and urine NGAL as an early biomarker of acute kidney injury in septic patients.

3. Patients and Methods:

This is a prospective, observational, cohort study carried out in the medical and surgical ICU, Mansoura Emergency Hospital, Mansoura University, in which we enrolled 40 patients with sepsis and septic shock.

Sepsis and septic shock were defined according to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [15].

- Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.
- Organ dysfunction can be identified as an acute change in total SOFA score ≥2 points consequent to the infection.
- The baseline SOFA score can be assumed to be zero in patients not known to have preexisting organ dysfunction.
- Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality.
- Patients with septic shock can be identified with a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain MAP ≥65 mm Hg and having a serum lactate level >2 mmol/L (18mg/dL) despite adequate volume resuscitation.

Acute kidney injury (AKI) was defined according to the KDIGO definition of 2012 [16] as an increment of serum creatinine ≥ 0.3 mg/dl within 48 h or an increase ≥ 50% from baseline within 7 days or urine output < 0.5 ml/kg/h for more than 6 h despite fluid resuscitation when applicable

Excluded from the study:

- Patients with chronic renal impairment.
- Prior kidney transplant.
- Renal replacement therapy (RRT) prior to ICU admission.
- Obstructive etiology for AKI.
- Patients with end stage renal disease (ESRD) on chronic renal replacement therapy.
- Patients with pre-existing AKI at the onset of shock
- Patients in whom the time of onset of shock started before hospital admission or could not be accurately determined.
- Patients with malignancies.

Institutional Review Board approval was obtained from the Research Ethics Committee of Cairo University.

Informed consent was obtained from all participants included in the study.

All participants included in the study were subjected to the following:

a) Detailed history taking and clinical examination

b) Routine laboratory investigations including :

Complete Blood Count (CBC), serum creatinine and Blood Urea Nitrogen (BUN), Liver Function Tests (LFT), Arterial Blood Gases (ABG) , serum sodium and potassium and blood lactate concentration.

c) Imaging study : Abdominal ultrasound

d) Calculation of APACHE II score on admission

e) Calculation of SOFA score daily.

f) **Measurement of plasma and urine NGAL levels:**

For patients with sepsis or septic shock , blood samples and urine samples were collected on enrollment and after 12 hours.

Reagent kit :

Quantikine® ELISA Human Lipocalin-2/NGAL Immunoassay.

Sample Collection and Storage:

- Plasma samples were collected using heparin as an anticoagulant, centrifuged for 15 minutes at 1000 x g within 30 minutes of collection and stored at≤ -20 °C.
- Urine samples were collected directly into a sterile container, centrifuged to remove particulate matter and stored at ≤ -20 °C.

Sample Preparation:

- Plasma samples required a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5-24 (diluted 1:5).
- Urine supernatant samples didn't require dilution as per manufacturer's instructions.

Reagent Preparation:

- The Human Lipocalin-2 Conjugate remained at 2-8 °C during use; all other reagents were brought to room temperature before use.
- Buffer was washed, when crystals formed in the concentrate, it was warmed to room temperature and mixed gently until the crystals had completely dissolved.
- 20 mL of Wash Buffer Concentrate were added to distilled water to prepare 500 mL of Wash Buffer.
- Calibrator Diluent RD5-24 (diluted 1:5) 20 mL of Calibrator Diluent RD5-24 Concentrate were added to 80 mL of distilled water to prepare 100 mL of Calibrator Diluent RD5-24 (diluted 1:5).
- Substrate Solution: Color Reagents A and B were mixed together in equal volumes within 15 minutes of use; protected from light. 200 µL of the resultant mixture was required per well.
- Human Lipocalin-2 Standard: the Human Lipocalin-2 Standard was reconstituted with 1.0 mL of distilled water. This reconstitution produced a stock solution of 100 ng/mL.

- The standard was mixed and allowed to sit for a minimum of 15 minutes. 900 µL of Calibrator Diluent RD5-24 (diluted 1:5) were pipetted into the 10 ng/mL tube. 500 µL of Calibrator Diluent RD5-24 (diluted 1:5) were pipetted into the remaining tubes
- The stock solution was used to produce a dilution series. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5-24 (diluted1:5) serves as the zero standard (0 ng/mL).

This assay employs the quantitative enzyme immunoassay technique. A monoclonal antibody specific for human Lipocalin-2 had been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any Lipocalin-2 present was bound by the immobilized antibody. An enzyme-linked monoclonal antibody specific for human Lipocalin-2 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of Lipocalin-2 bound in the initial step. The color development was stopped and the intensity of the color is measured.

Assay Procedure:

The procedure was performed according to the manufacturer's instructions listed below:

1) All reagents, working standards, and samples were prepared.
2) Excess microplate strips were removed from the plate frame, returned to the foil pouch containing the desiccant pack, and resealed.
3) 100 µL of Assay Diluent RD1-52 were added to each well; mixed well before and during use to avoid precipitate.
4) 50 µL of Standard, control, or sample were added per well; covered with the adhesive strip provided and incubated for 2 hours at 2-8 °C.
5) Each well was aspirated and washed; the process was repeated three times for a total of four washes. Washing was performed by filling each well with Wash Buffer (400 uL) using an auto-washer (Hydroflex TECAN). Complete removal of liquid at each step was done to ensure good performance. After the last wash, any remaining Wash Buffer was removed by aspirating; the plate was inverted and blotted against clean paper towels.
6) 200 uL of cold Human Lipocalin-2 Conjugate were added to each well; cover with a new adhesive strip and incubated for 2 hours at 2-8^{o}C.
7) The aspiration/wash was repeated as in step 5.
8) 200 uL of Substrate Solution were added to each well; incubate for 30

minutes at room temperature and protected from light.

9) 50 uL of Stop Solution were added to each well. The color in the wells changed from blue to yellow. When the color in the wells was green or the color change did not appear uniform, the plate was gently tapped to ensure thorough mixing.
10) Determine the optical density of each well was determined within 30 minutes, using a microplate reader (Infinite F50 TECAN).

All patients were followed up during their ICU course for development of AKI (primary end point).

Statistical analysis:

Data obtained from the present study were computed using SPSS versions 17 under the platform of Microsoft Windows 7. Continuous data were expressed in the form of mean ± SD while categorical data were expressed in the form of count and percent. Comparison of continuous data was performed utilizing student t test, while categorical data were done using Chi-square test. Relation between variables was investigated by Pearson's correlation coefficient. P value less than 0.05 was considered statistically significant.

4. Results:

Forty critically ill patients with sepsis and septic shock were enrolled in the study. Demographic data, baseline clinical characteristics and ICU outcome summarized in table (1). The mean age was 54.7 ± 12.2 years, 55% were males, and the mean BMI was 27.3 ± 4.2 kg/m². The mean APACHE II score was 18.8 ± 3.2, mean SOFA score was 6.9 ± 0.7, 75% required mechanical ventilation, 22.5% required vasopressors, 32.5% received diuretics, and 15% required renal replacement therapy. The most frequent source of sepsis was pulmonary infection (72.5%) **(Table2).**

Table (1) Demographic data, clinical characteristics and ICU outcome of the study group

Parameter	Study population (n=40) Mean ± SD
Age, years	**54.7 ± 12.2**
Gender, (male), n (%)	**22 (55%)**
BMI (kg/m²)	**27.3 ± 4.2**
Comorbidities, n (%)	
Cardiac disease	**22 (55%)**
COPD	**21 (52.5%)**
Diabetes Mellitus	**22 (55%)**

Hypertension	**13 (32.5%)**
Liver disease	**13 (32.5%)**
Cerebrovascular stroke	**7 (17.5%)**
Hemodynamic parameters	
HR	**122.7 ± 23.2**
SBP (mmHg)	**106.8 ± 19.4**
DBP (mmHg)	**59.5 ± 12.3**
Therapeutic interventions, n (%)	
Vasopressors	**9 (22.5%)**
Diuretics	**13 (32.5%)**
Mechanical ventilation	**30 (75%)**
Renal replacement therapy	**6 (15%)**
APACHE II	**18.8 ± 3.4**
SOFA	**6.9 ± 0.7**
Length of ICU stay , days	**15.7 ± 11.1**
Mortality, n (%)	**6 (15%)**

APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; SOFA, Sequential Organ Failure Assessment.

Table (2) Characteristics of sepsis in the study population

Parameter	**N = 40** **Mean ± SD**
Source of sepsis	
Pulmonary infection	**29 (72.5%)**
Intra abdominal infection	**8 (20%)**
Urinary infection	**2 (5%)**
Skin and soft tissue infection	**1 (2.5%)**
Isolated organisms	
No growth	**19 (47.5%)**
Echerishia Coli	**5 (12.5%)**
Gram-negative rods sputum	**4 (10%)**
Staphylococcus	**7 (17.5%)**
Streptococcus	**3 (7.5%)**
Pseudomonas	**2 (5%)**

Seventeen (42.5%) patients had AKI. Patients with AKI, compared to those without, had significantly higher APACHE II score (20.2 ± 3 vs. 17.7 ± 3.2, p = 0.02), SOFA score (7.4 ±

0.8 vs. 6.6 ± 0.7, p =0.002), more likely required vasopressors (p = 0.01) and renal replacement therapy (p = 0.002), more length of ICU stay (p = 0.01), and more mortality (p = 0.02) (Table 3). AKI patients had more positive bacterial growth on cultures (p = 0.01) **(Table 4).**

Table (3) Demographic data, clinical characteristics and ICU outcome of patients with AKI compared to those without

parameter	**AKI (n = 17) mean ± SD**	**No AKI (n = 23) mean ± SD**	**P**
Age, years	55.1 ± 13.6	54.4 ± 11.4	**0.87**
Gender, (male), n (%)	9 (52.9%)	13 (56.5%)	**0.82**
BMI (kg/m²)	27 ± 3.1	27.5 ± 5.1	**0.74**
Comorbidities, n (%)			
Cardiac disease	9 (52.9%)	13 (56.5%)	**0.82**
COPD	10 (58.8%)	11 (47.8%)	**0.49**
Diabetes Mellitus	7 (41.2%)	15 (65.2%)	**0.13**
Hypertension	6 (35.3%)	7 (30.4%)	**0.74**
Liver disease	6 (35.3%)	7 (30.4%)	**0.74**
Cerebrovascular stroke	3 (17.6%)	4 (17.4%)	**0.98**
Hemodynamic parameters			
HR	124.4 ± 21.1	121.4 ± 25.1	**0.69**
SBP (mmHg)	105.6 ± 20.5	107.8 ± 19.1	**0.72**
DBP (mmHg)	58.8 ± 12.2	60 ± 12.8	**0.77**
Therapeutic interventions, n			
Vasopressors	7	2	**0.01**
Diuretics	7	6	**0.3**
Mechanical ventilation	14	16	**0.3**
Renal replacement therapy	6	0	**0.002**
APACHE II	20.2 ± 3	17.7 ± 3.4	**0.02**
SOFA	7.4 ± 0.8	6.6 ± 0.7	**0.002**
Length of ICU stay , days	20.8 ± 14.5	11.8 ± 5.4	**0.01**
Mortality, n	5	1	**0.02**

AKI, acute kidney injury; APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; SOFA, Sequential Organ Failure Assessment.

Table (4) Characteristics of sepsis in the study groups

Parameter	AKI (n = 17) Mean ± SD	No AKI (n =23) Mean ± SD	P
Source of sepsis			
Pulmonary infection	15	14	**NS**
Intra abdominal infection	2	6	**NS**
Urinary infection	0	2	**NS**
Skin and soft tissue infection	0	1	**NS**
Isolated organisms			
Positive growth	14	7	**0.01**

AKI, acute kidney injury; NS, non significant.

Baseline laboratory values of the patient groups were summarized in **Table (5).**

Baseline laboratory values were not significantly different between the groups.

Table (5) Laboratory data of the study groups

	All (n = 40)	AKI (n = 17)	No AKI (n = 23)	P
Hemoglobin (g/dl)	10.7 ± 1.3	10.5 ± 1.8	10.8 ± 0.8	**0.49**
Leucocytes (10^3 /µl)	20.3 ± 2.5	20.3 ± 2.5	20.3 ± 2.7	**0.95**
Platelets (10^3 /µl)	234.5 ± 38.4	240.5 ± 36.9	230.0 ± 39.9	**0.40**
Sodium (mmol/L)	134.8 ± 6.4	135.2 ± 4.3	134.5 ± 7.8	**0.73**
Potassium (mmol/L)	3.7 ± 0.5	3.7 ± 0.6	3.7 ± 0.5	**0.98**
PaCO2 (mmHg)	35.8 ± 6.5	36.4 ± 7.5	35.4 ± 5.8	**0.63**
PaO2 (mmHg)	64.8 ± 17.4	67.0 ± 18.9	63.2 ± 16.6	**0.49**
Bicarbonate (mmol/L)	22.70 ± 5.95	23.12 ± 5.78	22.39 ± 6.18	**0.71**
AST(U/L)	71.63 ± 34.18	77.71 ± 38.86	67.13 ± 30.39	**0.34**
ALT(U/L)	52.25 ± 23.78	58.24 ± 22.98	47.83 ± 23.88	**0.17**
Bilirubin (mg/dl)	1.46 ± 0.90	1.66 ± 1.05	1.32 ± 0.75	**0.24**
Albumin(g/dl)	2.72 ± 0.44	2.69 ± 0.45	2.75 ± 0.43	**0.67**
Lactate (mmol/L)	2.95 ± 0.99	2.90 ± 1.07	2.99 ± 0.95	**0.79**
Creatinine (mg/dl)	0.81 ± 0.12	0.80 ± 0.12	0.82 ± 0.12	**0.57**
BUN (mg/dl)	**27.60 ± 12.05**	**25.94 ± 12.54**	**28.83 ± 11.80**	**0.46**

AKI, acute kidney injury; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen.

AKI patients, compared to those without, had significantly higher baseline plasma NGAL (221.4 ± 104.7 vs. 129.5 ± 48, p = 0.003), and at 12 h (239.2 ± 122.9 vs. 128.9 ± 49.1, p = 0.002). Similarly, significantly higher urine NGAL was observed in AKI patients at baseline (13.6 ± 5.5 vs. 7.8 ± 3, p = 0.001), and at 12 h (23.2 ± 6.5 vs. 15 ± 4.9, p = 0.0001) **(Table 6).**

Table (6) Comparison of the mean plasma and urine NGAL in the study groups

	All (n = 40) mean ± SD	**AKI (n =17) mean ± SD**	**No AKI (n =23) mean ± SD**	**P [a]**
Plasma NGAL, ng/ml	168.6 ± 88.9	221.4 ± 104.7	129.5 ± 48	**0.003**
Baseline	175.8 ± 103	239.2 ± 122.9	128.9 ± 49.1	**0.002**
At 12 hours				
Urine NGAL, ng/ml				
Baseline	10.3 ± 5.1	13.6 ± 5.5	7.8 ± 3	**0.001**
At 12 hours	18.5 ± 6.9	23.2 ± 6.5	15 ± 4.9	**0.0001**

[a] *, comparison between AKI and non-AKI groups; AKI, acute kidney injury; NGAL, neutrophil gelatinase associated lipocalin*

Baseline plasma NGAL showed a correlation with age (r = 0.43, p = 0.005), SOFA score (r = 0.42, p = 0.006), and length of ICU stay (r = 0.35, p = 0.02). Similarly, plasma NGAL at 12 h showed a correlation with age, SOFA score, length of ICU stay, and moreover, with APACHE II score (r = 0.31, p = 0.04). However, urine NGAL only at 12 h showed correlation with APACHE II score (r = 0.42, p = 0.005), SOFA score (r = 0.29, p = 0.01), and ICU length of stay (r = 0.36, p = 0.02) **(Table 7).**

Table (7) Association between plasma and urine NGAL and demographic data, severity scores and length of ICU stay

	Baseline pNGAL		**12-h pNGAL**		**Baseline uNGAL**		**12-h uNGAL**	
	r	P	r	P	r	P	r	**P**
AGE, years	**0.43**	0.005	**0.38**	0.01	0.04	0.7	0.24	**0.13**
BMI (kg/m²)	-0.1	0.5	0.05	0.7	-0.13	0.4	-0.01	**0.9**
APACHE II	0.26	0.1	**0.31**	0.04	0.14	0.38	**0.42**	**0.005**
SOFA	**0.42**	0.006	**0.4**	0.009	0.24	0.13	**0.29**	**0.01**
Length of ICU stay	**0.35**	0.02	**0.37**	0.01	0.22	0.17	**0.36**	**0.02**

APACHE, acute physiology and chronic health evaluation; BMI, body mass index; NGAL, neutrophil gelatinase associated lipocalin; SOFA, sequential organ failure assessment

Baseline plasma NGAL was significantly higher in patients required vasopressors, mechanical ventilation, and renal replacement therapy, likewise, both plasma NGAL at baseline and 12 h and baseline urine NGAL were significantly higher in patients who died **(Table 8).**

Table (8) Association between plasma and urine NGAL and gender, therapeutic interventions and mortality.

	pNGAL baseline	**P**	**pNGAL 12-h**	**P**	**uNGAL baseline**	**P**	**uNGAL 12-h**	**P**
Gender								
Male	173 ± 97	0.71	156 ± 91	0.19	10 ± 5.2	0.99	18 ± 6.5	**0.67**
Female	162 ± 79		199 ± 113		10 ± 5.1		19 ± 7.4	
Vasopressors								
Yes	266 ± 120	**0.01**	250 ± 116	**0.01**	14.7 ± 5.9	**0.002**	22.5 ± 7.4	**0.04**
No	140 ± 52		154 ± 89		9 ± 4.1		17.3 ± 6.4	
Mechanical ventilation								
Yes	184 ± 95	**0.05**	189 ± 113	0.15	11.1 ± 5.3	0.07	20.2 ± 6.7	**0.002**
No	121 ± 41		135 ± 49		7.8 ± 3.3		13.3 ± 4.6	
Diuretics								
Yes	198 ± 97	0.13	196 ± 106	0.39	10.4 ± 5.4	0.89	20.5 ± 7.1	**0.2**
No	154 ± 82		166 ± 101		10.2 ± 5		17.5 ± 6.7	
RRT								
Yes	287 ± 115	**0.03**	243 ± 124	**0.08**	17.9 ± 2.1	**0.001**	22.6 ± 7.1	**0.12**
No	147 ± 65		163 ± 96		9 ± 4.3		17.8 ± 6.8	
Death								
Yes	278 ± 124	**0.001**	272 ± 139	**0.01**	16.3 ± 3.6	**0.001**	22.8 ± 8.2	**0.1**
No	149 ± 66		158 ± 87		9.2 ± 4.6		17.7 ± 6.5	

NGAL, neutrophil gelatinase associated lipocalin; RRT, renal replacement therapy

At enrollment into two groups, AKI and non-AKI, plasma and urine NGAL at baseline and at 12 h showed good correlation with serum creatinine, however, there is no observed correlation with BUN **(Table 9).**

Table (9) Relation between plasma and urine NGAL levels and Kidney function at enrollment into two groups, AKI and non AKI

	pNGAL (Baseline)		pNGAL (12 h)		uNGAL (baseline)		uNGAL (12 h)	
	r	p	r	p	r	p	r	p
Creatinine (mg/dl)	**0.59**	**0.001**	**0.53**	**0.001**	**0.59**	**0.001**	**0.61**	**0.001**
BUN (mg/dl)	**-0.09**	**0.56**	**-0.3**	**0.055**	**0.1**	**0.5**	**0.06**	**0.7**

BUN, blood urea nitrogen; NGAL, neutrophil gelatinase associated lipocalin

We could observe that, the higher the baseline plasma and urine NGAL, the earlier the development of AKI **(Table 10).**

Table (10) Association between plasma and urine NGAL and the onset of AKI at different time points.

	AKI 12 h (n = 3)	AKI 24 h (n = 9)	AKI 48 h (n = 5)	Non-AKI (n = 23)	P-value
Plasma NGAL					
Baseline	391.3 ± 13.6	202 ± 87.3	164.8 ± 21	127.3 ± 46.4	**0.001**
12 h	259.3 ± 120	286.5 ± 132.9	142.2 ± 27.8	128.9 ± 49.1	**0.001**
Urine NGAL					
Baseline	18.7 ± 0.7	13.1 ± 5.9	11.5 ± 5.3	7.8 ± 3	**0.001**
12 h	24.1 ± 5.2	22.3 ± 7.5	24.1 ± 6.6	15 ± 4.9	**0.001**

AKI, acute kidney injury; NGAL, neutrophil gelatinase associated lipocalin

Analysis of the ROC curves showed that, a baseline pNGAL ≥ 147 ng/ml was associated with a sensitivity of 82.4% and specificity of 73.9% for predicting AKI in septic patients **(Table 11, Fig. 1),** 12 h pNGAL ≥ 142 ng/ml was associated with a sensitivity of 82.4% and specificity of 60.9% (Fig. 2), baseline uNGAL ≥ 7.45 ng/ml was associated with a sensitivity of 76.5% and specificity of 60.9% (Fig. 3), and 12 h uNGAL ≥ 19 ng/ml was associated with a sensitivity of 82.4% and specificity of 78.3% **(Fig. 4).**

Table (11) Diagnostic value of plasma and urine NGAL for predicting development of AKI

	AUC	P	Cut-off value	Sensitivity	Specificity
Plasma NGAL, ng/ml	0.82	0.001	147	82.4 %	**73.9 %**
Baseline	0.80	0.001	142	82.4 %	**60.9 %**
12 h					
Urine NGAL, ng/ml					
Baseline	0.79	0.001	7.45	76.5 %	**60.9 %**
12 h	0.82	0.001	19.0	82.4 %	**78.3 %**

NGAL, neutrophil gelatinase associated lipocalin

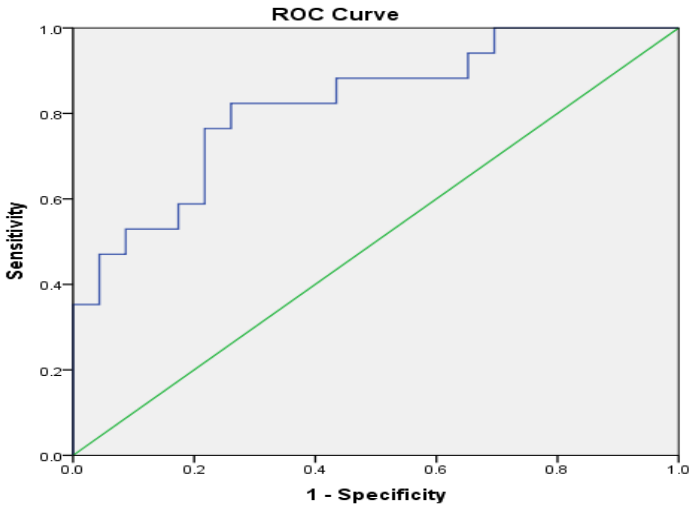

Figure (1) Diagnostic value of baseline pNGAL to predict AKI

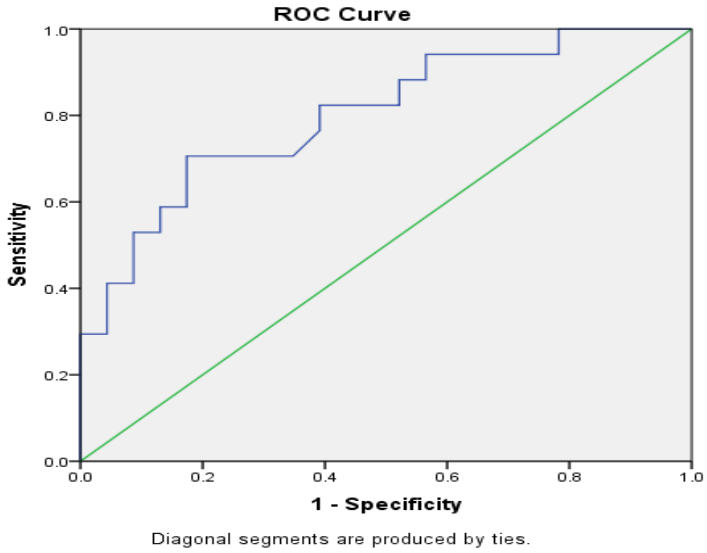

Figure (2) Diagnostic value of pNGAL at 12 h to predict AKI

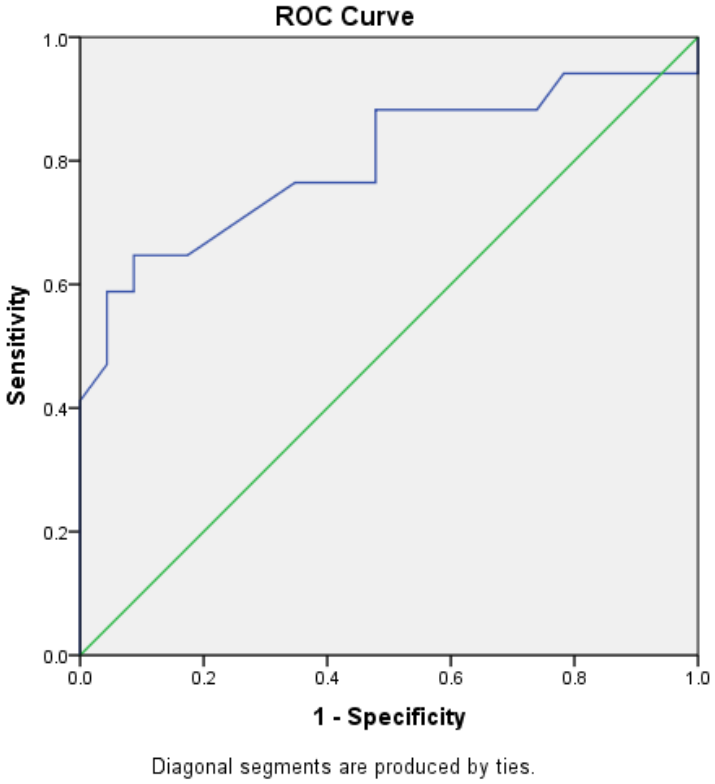

Figure (3) Diagnostic value of baseline uNGAL to predict AKI

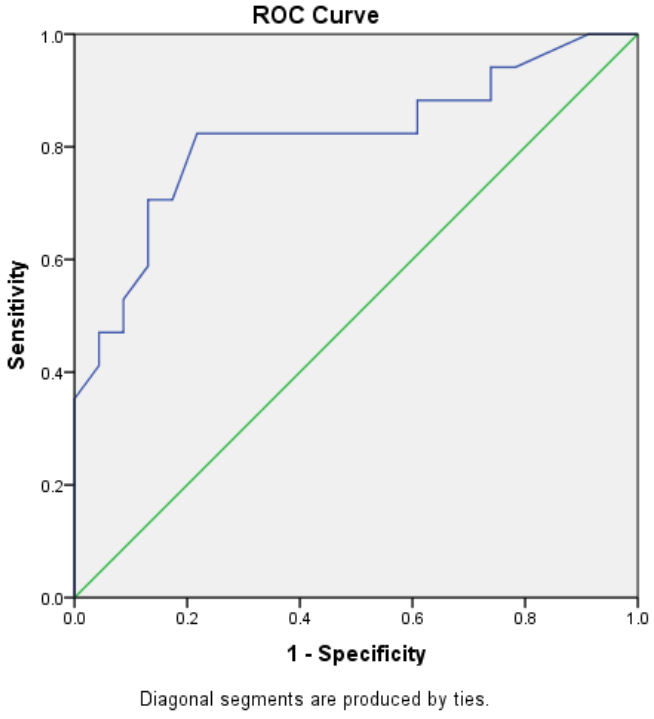

Figure (4) Diagnostic value of uNGAL at 12h to predict AKI

5. Discussion:

Acute kidney injury (AKI) includes a spectrum of clinical manifestations ranging from mild injury to severe damage [17]. Several studies have analyzed the etiology of acute renal failure and have found that sepsis is a key contributing factor in AKI patients who are admitted to intensive care units (ICUs).Scientific evidence has indicated that 35%-50% of critically ill patients with AKI have renal injury due to sepsis [18]. Patients who are diagnosed with septic AKI have a higher mortality risk than patients with non-septic AKI. In septic AKI patients, survival is associated with longer ICU and hospital stays [19]. Conversely, studies that initially focused on sepsis reported that 10%-50% of patients with sepsis subsequently developed AKI [20]. Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family of proteins. Furthermore, NGAL has emerged as a potentially useful diagnostic biomarker in acute kidney injury (AKI) [21]. However, it was demonstrated that NGAL is also a marker of bacterial infection and systemic inflammation [22]. In the present study , we enrolled 40 critically ill patients with sepsis and septic shock. In our study 17 (42.5%) patients developed AKI. Meanwhile this rate is lower than reported by the study of ***Suh et al.,*** [23] who reported that AKI was developed in 57.7% of 992 patients. Also, in the study of ***Feng et al.,*** [24], 54.2% of 107 patients with severe sepsis and septic shock had AKI. Comparison between patients with AKI and patients without regarding the demographic data revealed no statistically significant differences regarding age, BMI and sex distribution. This is in accordance with results obtained by the study of ***Wang et al.,*** [25] who studied AKI in 211 septic patients admitted to the intensive care unit (ICU) and showed patients with AKI and patients without had similar age and sex distribution. On the contrary to our results, populations at high risk of sepsis associated-AKI have been identified. Elderly patients carry a higher incidence rate of sepsis associated-AKI [26]. In addition, females were found to be affected more commonly. Baseline comorbidities, specifically chronic kidney disease, diabetes mellitus, heart failure, malignancy, and liver disease; all increase patients′ susceptibility to AKI [27]. In our study there were no statistically significant differences between patients with AKI and patients without regarding the underlying co-morbidities. However, ***Bagshaw et al.*** [28] found that septic AKI patients,

compared to those with non septic AKI had more co morbid illness (P=0.005) including patients with malignancies (p=0.002). Also, there were no statistically significant difference between patients with AKI and patients without regarding the admission hemodynamic parameters. This is in agreement with the study of ***Plataki et al.,*** [29] who aimed determine the risk factors associated with AKI development in patients with septic shock. In their study comprising 390 patients, patients with and without AKI had comparable blood pressure levels. Furthermore, in our study no statistically significant differences were found between patients with and without AKI regarding the baseline laboratory data in agreement with the study of ***Feng et al.*** **[**24]. Regarding the microbial data, we could observe that patients with AKI had significantly higher frequency of positive isolated organisms when compared with patients without AKI. This is accordance with the study of ***Suh et al.,*** [23]. In their study on 992patients with sepsis and septic shock, patients with AKI had significantly higher frequency of positive blood culture results. In respect to NGAL levels, we found significantly higher plasma and urine NGAL levels at baseline and at 12 hour follow up period in patients with AKI when compared with patients without. This is in agreement with the study of ***Katagiri et al.*** [30] who found that septic patients with AKI have significantly higher NGAL levels when compared with septic patients without AKI . Also, the study of ***Pickering and Endre*** [31] who found that plasma NGAL was significantly increased in ICU patients with AKI in comparison to patients without. The study of ***Camou et al.*** [32] found that pNGAL concentration was significantly higher in patients with AKI compared to patients without AKI (471ng/mL versus 134ng/mL, P<0.001). Moreover, the study of ***de Geus et al.,*** [33] found that plasma NGAL levels were increased in septic and non-septic patients with AKI when compared with patients without AKI. Furthermore, ***Md Ralib et al.*** [34] found that serum NGAL is significantly increased in AKI patients with and without sepsis when compared with patients without AKI. In addition, ***Cho et al.*** [35] studied serum and urinary NGAL levels in septic-AKI, septic-non AKI and non-septic AKI groups. The study found that serum and urine NGAL levels were significantly elevated in patients with septic AKI group. Also, in the study of ***Vanmassenhove et al.,*** [36] uNGAL and sNGAL were measured at admission (T0) and 4 hours (T4) and 24 hours later (T24) in patients with sepsis. The study found that at all time points both markers were increased in patients with AKI in comparison with

patients without. Similar results were obtained by the study of ***Dai et al.*** [37]. Notably, in the study of ***Nga et al.,*** [38] urine was analyzed for NGAL within the first 24 hours after admission (classified as NGAL1), between 24 and 48 h (NGAL2), and at moment of AKI diagnosis (NGAL3). The study found that urinary NGAL levels were significantly higher in AKI patients when compared with non-AKI patients at all time points. The value of NGAL in the diagnosis of AKI in septic patients was confirmed by a recent meta analysis concluding that NGAL is not only an effective predictive factor for AKI in the process of sepsis, but also shows potential predictive value for RRT and mortality [39]. Another systematic review declared similar results but it was limited to plasma NGAL only [40]. However, in the study of ***Mårtensson et al.,*** [41] the investigators studied the impact of inflammation/sepsis on the concentrations of NGAL in plasma and urine in adult intensive care unit (ICU) patients. Of the 45 patients included in the study, 40 had elevated peak levels of pNGAL. Peak levels of pNGAL were not significantly different between septic shock patients with and without AKI. In another study, ***Aydoğdu et al.*** [42] found that patients with sepsis-AKI had significantly higher urinary but not serum NGAL when compared with patients without AKI. On the other hand, ***Nisula et al.*** [43] noted that urinary NGAL failed to predict AKI in critically ill patients. Comparison between patients with and without AKI regarding the severity scores had shown that patients with AKI had significantly higher APACHE II scores in agreement with the study of ***Vanmassenhove et al.*** [36]. In 2020, Tornblom et al. [44] studied the clinical usefulness of urine NGAL as a biomarker for acute kidney injury in 484 critically ill patients with sepsis . Their study concluded that the performance of uNGAL was inadequate in predicting AKI and do not support the use of uNGAL in critically ill septic patients to predict AKI or clinical outcomes. In contrast, the study of Zhou H et al. in 2021 [45] urine NGAL demonstrated high performance as diagnostic biomarker of AKI in septic patients. Recently, Chen Y et al in 2022 [46] investigated the value of serum NGAL (sNGAL), urinary NGAL (uNGAL), KIM-1, and IL-18 for the early clinical diagnosis of sepsis-induced AKI in a cohort of 100 pateints .They found that sNGAL, uNGAL, KIM-1 and IL-18 can be used as early indicators for the early diagnosis of sepsis-induced AKI, and they will increase and maintain at a higher level as the disease progresses. Also, we found that patients with AKI had significantly higher SOFA scores in agreement with the study of ***Bojic et al.*** [47]. In the present

study, patients with AKI had significantly higher frequency of administration of vasoactive drugs and use of RRT in accordance with the study of ***Suh et al***. [23]**.** Regarding the outcome, we found that patients with AKI had significantly higher mortality rate and longer length of hospital stay when compared with patients without AKI. This is in line with data reported by the study of ***Tu et al.*** [48]. In the present study regarding relation between serum and urinary NGAL values by timing of AKI showed that, serum NGAL at baseline and at 12 h were higher among patients who had AKI at 12 hour; the mean measurement (391.3, 259.3 ng/ml respectively) (p=<0.001) than patients who developed AKI within 24 hours (202,286.5ng/ml respectively),whereas those who developed AKI within 48 hour had mean of(164.8, 142.2ng/ml respectively) this is in agreement with the data reported by ***Cruz DNet al.***[49], they found pNGAL was a good independent predictor for the development of AKI within the next 48 h (AuROC 0.78, 95% CI 0.65–0.90) and for RRT use during the ICU stay (AuROC 0.82, 95% CI 0.70–0.95). In our study both serum and urinary NGAL were good predictors of AKI. The ability of serum NGAL at the baseline and 12hour follow up period to predict AKI in patients with sepsis showed AUC-ROC(0.82,0.80respectively),Sensitivity(82.4%,82.4%respectively) and specificity (73.9%,60.9%respectively). The ability of urinary NGAL at the baseline and 12hour follow up period to predict AKI in patients with sepsis showed AUC-ROC (0.79,0.82 respectively), sensitivity (76.5%, 60.9% respectively) and specificity (73.9%,78.3% respectively). In the study of ***Mårtensson et al.*** [41], they found both pNGAL and uNGAL were good predictors of AKI within the next 12 h. However, the ability of pNGAL to predict AKI in patients with septic shock was poor and the use of uNGAL is more useful in predicting AKI as the levels are not elevated in septic patients without AKI [50,51]. AUC-ROC of urinary NGAL varied from 0.74 [52] to 0.88 [53]. The non-consistent performance across studies in critically ill adult patients can be explained by several factors. First, comparing a biomarker of assumed parenchymal kidney injury against a reference method (i.e. creatinine) that imperfectly estimates functional kidney impairment (clinical AKI) is problematic. Due to continuing loss of muscle mass in the critically ill [54] and dilution of serum creatinine fluid-loaded patients [55], AKI might go undetected by conventional creatinine-based criteria. Emerging evidence shows that patients with elevated NGAL in the absence of creatinine-based

criteria for AKI carry an increased risk of adverse events including need for renal replacement therapy and death [56, 57].Whether this specifically represents subclinical AKI or is simply an expression of severe systemic inflammation is yet to be determined. Second, the burden of comorbidities, especially chronic kidney disease (CKD), may affect the results since CKD per se is associated with elevated serum and urinary NGAL levels. ***Mitsnefes et al***. [58] and ***Bolignano etal.*** [59] found that NGAL only predicted AKI in patients with normal baseline renal function. The notion that comorbidities are important confounders in the studies is further supported by the fact that the predictive performance is generally better in children [51]. Third, the time from biomarker measurement to AKI diagnosis differs substantially among studies. It could be expected that predictive values would increase when NGAL is measured closer to the time of insult. ***Kashani et al***. [60] found only a limited performance (ROC area 0.69) when NGAL was used to predict severe AKI within 12 h. Finally, existing studies are affected by the limited ability of the NGAL assays to distinguish between the various molecular forms released by different tissues. Systemic inflammation triggered by conditions such as sepsis or procedures like cardiopulmonary bypass (CPB) is strongly associated with AKI development [18].

In conclusion, plasma and urine NGAL on admission and at 12 h showed a good performance in predicting AKI in a subset of critically ill patients with sepsis and septic shock.

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