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Evaluation of Urinary NGAL as a Diagnostic Tool for Acute Kidney Injury in Critically Ill Patients With Infection: An Original Study

Brenno Cardoso Gomes[1], João Manoel Silva Júnior[2], and Felipe Francisco Tuon[3]

Abstract

Background: Acute kidney injury (AKI) is a common complication in critical care patients. The presence of AKI is a marker for poor outcomes such as longer hospitalization durations, more hospital readmissions, and especially, higher mortality rates. Sepsis is one of the major causes of AKI within the intensive care unit (ICU) population. Sepsis-related AKI occurs in approximately 20% of patients, reaching more than 50% in patients with septic shock. The diagnosis of AKI depends on urine output and/or serum creatinine measurements. Unfortunately, serum creatinine is a late and unreliable (insensitive and nonspecific) indicator of AKI. However, biomarkers of renal damage have great potential in facilitating early diagnosis of AKI. Several biomarkers, including urinary neutrophil gelatinase-associated lipocalin (uNGAL), have been used in the early detection of AKI.

Objectives: The aim of this study was to evaluate uNGAL for the diagnosis and prognosis of AKI in critical ill patients with infections.

Design: Original study (Cohort Prospective Observational).

Setting: Study in 2 ICUs of different Brazilian hospitals, in the city of Curitiba: Hospital de Clínicas da Universidade Federal do Paraná and Hospital da Polícia Militar do Paraná, from November 12, 2016 to May 15, 2018.

Participants: Critically ill patients with infections, sepsis, or septic shock were selected. The inclusion criteria were patients older than 18 years with infection. They were followed up for 30 days in the analysis of outcomes. We requested that consent forms be signed by all eligible patients or their caregivers.

Measurements: The urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels of the patients were measured on 4 consecutive days and was assayed using a chemiluminescent microparticle immunoassay system. The screening time occurred within 72 hours of admission to the ICU. The first urine sample was collected within the first 24 hours of the screening hours. Mortality and AKI were assessed during first 30 days.

Methods: clinical and laboratory data, including daily uNGAL levels, were assessed. The AKI stage using the KDIGO criteria was evaluated. Sensitivity, specificity, and the area under the curve-receiver operating characteristic (AUC-ROC) values were calculated to determine the optimal uNGAL level for predicting AKI.

Results: We had 38 patients who completed the study during the screening period. The incidence of AKI was 76.3%. The hospitalization period was longer in the group that developed AKI, with 21 days of median (interquartile range [IQR]: 13.5-25); non-AKI group had a median of 13 days (IQR 7-18; $P = .019$). We found a direct relationship between uNGAL levels and the progression to AKI. Increased values of the biomarker were associated with the worsening of AKI ($P < .05$). The cutoff levels of uNGAL that identified patients who would progress to AKI were the following: (d1) >116 ng/mL, (d2) >100 ng/mL, and (d3) 284 ng/mL. The value of the fourth and last measurement was not predictive of patients who would progress to AKI. The median urinary uNGAL was also associated with mortality on Days 1, 3, and 4: d1, $P = .039$; d3, $P = .005$; d4, $P = .005$. The performance of uNGAL in detecting AKI patients (AUC-ROC = 0.881). There were no risk factors other than AKI that could be correlated with increased uNGAL levels on Day 1.

Limitations: The study was carried out in 2 centers, having used only 1 biomarker, and our small number of patients were limitations.

Conclusion: the uNGAL had an association in its values with the diagnosis and prognosis of patients with severe infections and AKI. We suggest that studies with a greater number of patients could better establish the cutoff values of uNGAL and/or serum NGAL in the identification of infected patients who are at a high risk of developing AKI.

Abrégé

Contexte`: L'insuffisance rénale aiguë (IRA) est une complication fréquente chez les patients des unités de soins intensifs (USI). L'IRA est un marqueur d'issues défavorables pour ces patients, notamment d'hospitalisations plus longues, de réadmissions plus fréquentes et surtout, de taux de mortalité plus élevés. Le sepsis est une des principales causes d'IRA chez les patients soignés aux USI; cette infection liée à l'IRA survient chez environ 20 % des patients et peut toucher plus de 50 % des patients en choc septique. Le diagnostic de l'IRA repose sur la mesure de la diurèse ou du taux de créatinine sérique; cette dernière mesure s'avérant toutefois un indicateur tardif et peu fiable (non spécifique et peu sensible). Les biomarqueurs d'une lésion rénale pourraient potentiellement faciliter un diagnostic précoce de la maladie. Plusieurs, dont la NGAL urinaire ou uNGAL (*urinary neutrophil gelatinase-associated lipocalin*) ont déjà été utilisés dans ce contexte.

Objectifs: Évaluer le potentiel de la uNGAL pour le diagnostic et le pronostic de l'IRA chez les patients gravement malades souffrant d'infections.

Type d'étude: Étude initiale (étude de cohorte prospective et observationnelle).

Cadre: L'étude s'est tenue entre le 12 novembre 2016 et le 15 mai 2018 dans les USI de deux hôpitaux de Curitiba au Brésil (Hospital de Clínicas da Universidade Federal do Paraná et Hospital da Polícia Militar do Paraná).

Sujets: Les patients adultes, gravement malades et atteints d'une infection, d'un sepsis ou d'un choc septique ont été retenus. Le consentement écrit de tous les patients admissibles et de leurs représentants était exigé. Les sujets ont été suivis pendant 30 jours pour l'analyse des résultats.

Mesures: Les taux d'uNGAL ont été mesurés pendant quatre jours consécutifs et analysés par immunodosage microparticulaire par chimiluminescence. Le dépistage a eu lieu dans les 72 heures suivant l'admission aux USI et le premier échantillon d'urine a été prélevé dans les 24 premières heures de la période de dépistage. L'IRA et la mortalité ont été évaluées pendant les 30 premiers jours.

Méthodologie: L'analyse porte sur les données cliniques et de laboratoire, y compris les taux quotidiens d'uNGAL. Le stade de l'IRA a été établi selon les critères KDIGO. La sensibilité, la spécificité et les valeurs de surface sous la courbe ROC (SSC-ROC) ont servi à calculer le taux optimal d'uNGAL prédictif de l'IRA.

Résultats: L'incidence de l'IRA s'établissait à 76,3 % parmi les 38 patients ayant complété le dépistage. Les patients souffrant d'IRA étaient hospitalisés plus longtemps que les autres (durée médiane: 21 jours [ÉIQ: 13,5-25] contre 13 jours [ÉIQ: 7-18] pour les autres patients; p=0,019). Un lien direct entre le taux d'uNGAL et une progression vers l'IRA a été observé, et l'augmentation de ces valeurs a été associée à une aggravation de l'IRA ($p<0,05$). Les valeurs seuil d'uNGAL permettant de diagnostiquer une évolution vers l'IRA étaient les suivantes: (j1) $>$ 116 ng/mL; (j2) $>$ 100 ng/mL et (j3) 284 ng/mL. La valeur de la 4e et dernière mesure n'a pas permis de prédire une évolution vers l'IRA. Les taux médians d'uNGAL ont également été associés à la mortalité aux jours 1,3 et 4; avec des valeurs de *p* s'établissant à 0,039 (j1), 0,005 (j3) et 0,005 (j4). La performance du taux d'uNGAL pour détecter l'IRA (SSC-ROC) était de 0,881. Aucun facteur de risque autre que l'IRA n'a pu être corrélé avec une augmentation du taux d'uNGAL au jour 1.

Limites: L'étude ne s'est tenue que dans deux centres, sur un échantillon restreint de patients, et ne portait que sur un seul biomarqueur.

Conclusion: Le taux d'uNGAL a montré une association avec le diagnostic et le pronostic des patients souffrant d'infections graves et d'IRA. Nous pensons que des études sur un plus grand nombre de patients pourraient préciser les valeurs seuil d'uNGAL ou de NGAL sérique pour le dépistage des patients infectés qui présentent un risque élevé de développer une IRA.

Keywords

biomarkers urinary neutrophil gelatinase-associated lipocalin, acute kidney injury, sepsis, antibiotic, dialysis

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Introduction

The major causes of acute kidney injury (AKI) in the intensive care unit (ICU) include renal hypoperfusion, sepsis, and direct nephrotoxicity by drugs. However, in most cases, the pathogenesis is multifactorial, involving nonmodifiable factors (eg, age, comorbidities, and illness severity).[1,2] The

[1]Departamento de Medicina Integrada, Setor de Ciências da Saúde, Universidade Federal do Paraná, Curitiba, Brazil
[2]Departamento de Anestesiologia, Universidade de São Paulo, Brazil
[3]Escola de Medicina, Pontifícia Universidade Católica do Paraná, Curitiba, Brazil

Corresponding Author:
Brenno Cardoso Gomes, Departamento de Medicina Integrada, Setor de Ciências da Saúde, Universidade Federal do Paraná, R. Padre Camargo, 280–Alto da Glória, Curitiba 80060-240, Brazil.
Email: brennogomes@ufpr.br

presence of AKI is a marker for poor outcomes such as longer hospitalization durations, more hospital readmissions, and especially, higher mortality rates.[3-5]

Acute kidney injury in critically ill patients is independently associated with increased costs, morbidity, and mortality.[6] Sepsis is one of the major causes of AKI within the ICU population.[7] The pathophysiology of AKI caused by sepsis and the concept of AKI/sepsis interaction are topics of intense discussion.[8] Sepsis-related AKI occurs in approximately 20% of patients, reaching more than 50% in patients with septic shock. The combination of AKI and sepsis is associated with an approximately 70% mortality rate compared to the 45% mortality rate among patients with AKI alone.[9]

The diagnosis of AKI depends on urine output (UO) and/or serum creatinine (SCr) measurements. Unfortunately, SCr is a late and unreliable (insensitive and nonspecific) indicator of AKI.[7,10] However, biomarkers of renal damage have great potential in facilitating early diagnosis of AKI.[10] Neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein consisting of a polypeptide chain of 178 amino acids covalently bound to gelatinase. Urinary NGAL (uNGAL) can be derived from either the overflow of the systemic circulation, reduced reabsorption at the proximal tubular level, or increased production at the distal tubular level.

Moreover, NGAL can be produced by hematopoietic and nonhematopoietic cells. The renal proximal tubules synthesize and secrete uNGAL when AKI. The NGAL is released in the urine following ischemic or nephrotoxic insults.[11-14] The dynamic curve of uNGAL can help stratify the severity of renal damage and assist in the diagnostic and therapeutic decision-making process.[15]

The main objectives of this study are to analyze the role of urinary NGAL as a diagnostic tool for AKI in patients with different levels of infections, thus estimating some prognostic relationship with decreased renal function and mortality.

Methods

Study Design

This was a prospective observational study of critically ill patients with infections, sepsis, or septic shock in 2 ICUs of different Brazilian hospitals, which occurred from November 12, 2016 to May 15, 2018. Patient recruitment was performed at 2 hospitals in the city of Curitiba: a 562-bed public school hospital (Hospital de Clínicas da Universidade Federal do Paraná) and a 110-bed hospital (Hospital da Polícia Militar do Paraná), with an ICU specialized in the clinical and surgical care of military police officers. *The study was approved by the Local Ethics Committee (#58317216.2.0000.0096).*

Inclusion and Exclusion Criteria

The inclusion criteria were patients older than 18 years admitted to one of the 2 ICUs with the clinical diagnosis of infection and antimicrobial therapy. We requested that consent forms be signed by all eligible patients or their caregivers. The exclusion criteria were patients who received antimicrobial therapy for >24 hours from the beginning of the first biomarker assessment, those with chronic kidney disease in any stages, those with kidney transplants, pregnant and postpartum females, those undergoing renal replacement therapy (RRT), and those who experienced a cardiorespiratory arrest up to 72 hours before the first biomarker assessment.

Clinical Data

The following clinical and laboratory data were assessed: sex, age, outcome, lactate levels, vasoactive drugs, previous ICU admissions, indications for antibiotics, sites of infection, and concomitant nephrotoxic drug therapy during antibiotic use (vancomycin, loop diuretics, amphotericin B, polymyxin or colistin, nonsteroidal anti-inflammatory drugs, or contrast medium). Use of corticosteroids and several comorbidities were used to calculate the Charlson Index. Acute Physiology and Chronic Health Disease Classification System II (APACHE II) scores were calculated for all patients. Sequential Organ Failure Assessment (SOFA) and Quick SOFA (qSOFA) scores were also assessed on study inclusion day. Infection severity was classified according to Sepsis-3 criteria: infection (local infectious process without organ dysfunctions); sepsis (life-threatening organ dysfunction caused by a dysregulated host response to infection, suspected or overt infection, and acute increase of ≥2 points in SOFA scores in response to an infection, representing organ dysfunction); and septic shock (hypotension requiring vasopressors to maintain mean arterial pressure of >65 mmHg and having a serum lactate level of >2 mmol/L despite adequate volume resuscitation).[16,17]

The uNGAL levels of the patients were measured on 4 consecutive days and were assayed using a chemiluminescent microparticle immunoassay system (Abbott Laboratories Inc., Wiesbaden, Germany). The screening time occurred within 72 hours of admission to the ICU. The first urine sample was collected within the first 24 hours of the screening hours.

This analytical system measures the emission of chemiluminescence to quantify the level of NGAL in the sample being analyzed. The test is performed using the ARCHITECT i2000 SR (Abbott, Illinois, USA). The results range from 10 to 1500 ng/mL; however, in cases with results of >1,500 ng/mL, the system uses an automatic dilution protocol and is able to report results of up to 6,000 ng/mL using a 1:4 dilution of the sample. Urinary neutrophil gelatinase-associated lipocalin assessment was performed for all patients included in the study, regardless of infection severity classification. The first measurement was performed within 24 hours of a patient's screening, and the next 3 at regular intervals of 24 hours. After a bladder catheter was placed,

10 mL of urine were collected and sent to the laboratory. Tests were always performed on the same equipment by the same operator in the Hospital de Clínicas da Universidade Federal do Paraná in Curitiba, Brazil.

Definition of AKI

Daily SCr levels and UOs were assessed. The UO was collected invasively through a bladder catheter. The patients' lowest SCr levels measured before study inclusion were considered baseline. AKI was classified according to the Kidney Disease Improving Global Outcomes (KDIGO) criteria used in previous studies.[18-21]

Statistical Analysis

Qualitative data were described as percentages, and quantitative data were described as arithmetic mean or median value according to the distribution pattern. Standard deviation (SD) and interquartile ranges (IQRs) 25% and 75% (IQ) were distribution variables for mean and median, respectively. Risk factors associated with outcomes (death and AKI) were calculated according to each variable and its distribution, as determined by Student *t* test, Mann-Whitney *U*, chi-square, or Fisher exact test. A difference of under 5% ($P < .050$) was statistically significant. For the multivariate analysis, all variables with statistical significance in the univariate analysis were included in a binary logistic regression.

Sensitivity and specificity were calculated, and the area under the curve of a receiver operating characteristic (AUC-ROC) was considered as the optimal uNGAL cutoff level for predicting all classifications of AKI. Survival curves (Kaplan-Meier) were constructed from the time of antibiotic initiation to a patient's death or discharge. Overall mortality was included in the analysis and thirty-day mortality curves were constructed, and the Gehan-Breslow-Wilcoxon test was performed. All tests were performed with SPSS 23 (IBM, Armonk, NY, USA).

Results

During the study period, 46 patients were screened, and 8 were excluded: 1 patient was undergoing hemodialysis when screened, 2 patients had missing samples, 3 had unfilled infection criteria, 1 patient refused to sign the informed consent form, and 1 had an early death. Therefore, from the 46 patients, only 38 of them have complete data analysis (November 12, 2016 to May 15. 2018).

Regarding the quantitative variables analyzed during follow-up in the groups which presented some form of AKI versus without AKI, we found a few differences with statistical significance ($P < .05$) such as the hospitalization period was longer in the group that developed AKI, with 21 days of median (IQR: 13.5-25); non-AKI group had a median of 13 days (IQR: 7-18; $P = .019$). Table 1 shows baseline characteristics.

An analysis of the 2 groups (with AKI and without AKI) found 26.3% (n = 10) of patients with infections without severity criteria, 26.3% (n = 10) of patients with septic shock, and 47.3% (n = 18) with sepsis. Of the 10 cases found in patients who had infection without severity criteria, 60% (n = 6) had AKI at some point; among sepsis patients (18 cases), 77.7% developed AKI (n = 13); and 90% (n = 9) of patients who presented septic shock (10 cases) developed some level of AKI. None of the quantitative or qualitative variables were independent risk factors for AKI in the multivariate analysis.

Respiratory infection was the most frequently observed (n = 11; 29%), followed by skin and soft tissue (n = 9; 23.3%) and abdominal infections (n = 5; 13.1%). The most prescribed antibiotics were piperacillin/tazobactam (n = 12; 31.5%), meropenem (n = 8; 21%), and polymyxin B (n = 6; 15.7%). The median duration of antimicrobial treatment was 7.5 days (IQR: 7-11.5). We found no association between antibiotic use, either alone or in combination, and the development of acute renal injury. Similarly, use of contrast agents and nonsteroidal anti-inflammatories were not associated with AKI development.

The incidence of AKI was 76.3% (n = 29). The maximum KDIGO classification found in AKI patients was AKI stage 1 was present in 20.6% (n = 6) of patients, AKI stage 2 in 13.7% (n = 4), and AKI stage 3 in 65.5% (n = 19). Among patients who developed AKI, 20% (n = 6) underwent RRT; of these patients, 83.3% (n = 5) were classified as AKI stage 3, and the remainder (n = 1) as AKI stage 2. There was a statistical difference in the measurements of urinary NGAL in the 4 days (d1-d4) between the groups with AKI and without AKI (Table 2).

We found a direct relationship between uNGAL levels and the progression to AKI. Increased values of the biomarker were associated with the worsening of AKI ($P < .05$; Table 2). Figure 1 demonstrates the performance of uNGAL in detecting AKI patients (AUC-ROC = 0.881). The cutoff value of uNGAL for AUC-ROC was defined by the best value to reach the ideal sensitivity and specificity. This value was 200 ng/mL. The median of uNGAL level increased progressively with respect to the evolution of the KDIGO criteria for the first to fourth measurements taken (days; Table 3).

The cutoff levels of uNGAL that identified patients who would progress to AKI were the following: (d1) > 116 ng/mL; (d2) > 100 ng/mL, and (d3) 284 ng/mL. The value of the fourth and last measurement was not predictive of patients who would progress to AKI. Figure 2 presents the difference in the values of urinary NGAL in all days analyzed (d1-d4) in patients without AKI (9 patients), AKI without dialysis (23 patients), and AKI in RRT (6 patients). At Days 2, 3, and 4 measurement time points, patients with sepsis and septic shock presented higher uNGAL rates than did patients without organic dysfunctions (Figure 3).

Table 1. Baseline Characteristics of Patients.

	Without AKI (n = 9)		With AKI (n = 29)			
	n	%	n	%	*P* value	Multivariable analysis
Female	5	56	9	31	.174	
Male	4	44	20	69		
Mechanical ventilation	3	33	14	48	.346	
HIV	1	11	1	3	.422	
Diabetes	0	0	8	28	.088	NS
Heart failure	2	22	8	28	.506	
Peripheral arterial disease	3	33	10	35	.640	
COPD	2	22	10	35	.401	
SAH	2	22	18	62	.043	NS
Neoplasm	0	0	9	31	.061	NS
Rheumatic diseases	2	22	1	3	.134	
Peptic ulcer	0	0	6	21	.172	
Cirrhosis	0	0	2	7	.578	
Corticoid use	4	44	6	21	.163	
Immunosuppression	4	44	11	38	.510	
Trauma	1	11	2	7	.567	
Elective surgery	1	11	2	7	.567	
Emergency surgery	3	33	8	28	.522	
NSAIDs	2	22	2	7	.244	
Contrast	3	33	3	10	.131	
Infection	4	44	6	21	.163	
Sepsis	4	44	14	48	.573	
Septic shock	1	11	9	31	.233	
Vasoactive drugs day 0	1	11	10	35	.179	

Note. AKI = acute kidney injury; NS = not significant; COPD = chronic obstructive pulmonary disease; SAH = systemic arterial hypertension; NSAIDs = nonsteroidal anti-inflammatory drugs.

The overall mortality rate of the patients was 42.1% (n = 16). There was no difference in the mortality rate of patients with AKI 48.2% (n = 14) and those without AKI 22.2% (n = 2; Figure 4). Moreover, 83.3% (n = 5) of patients undergoing RRT died. According to the classification of infection severity, the mortality found was infected patients without organic dysfunction 20% (n = 2), patients with sepsis 38% (n = 10), and septic shock 70% (n = 7).

The median uNGAL was also associated with mortality on Days 1, 3, and 4: d1, $P = .039$; d3, $P = .005$; d4, $P = .005$). There were no risk factors other than AKI that could be correlated with increased uNGAL levels on Day 1. Multivariate analysis did not reveal any factors that were independently related to death.

Discussion

The determination of specific causes of AKI in critically ill patients remains challenging. The KDIGO definition for AKI is based on creatinine and oliguria, 2 imperfect markers.[22] Although creatinine and urinary output has been used for a long time as diagnostic forms AKI, they have only recently been used in a worldwide consensus. These markers were actually presented as ways to make the (consensual) diagnosis of AKI in 2004, during the Acute Dialysis Quality Initiative (ADQI). Thus, the RIFLE classification (Risk, Injury, Insufficiency, Loss, and End-stage renal disease) was the first criterion used as a consensus. In 2007, the AKIN criteria (Acute Kidney Injury Network) emerged, and in 2012, the KDIGO criteria, all aimed at standardizing the AKI diagnosis.[20,23,24]

Over the last few years, significant progress has been made in the field of novel biomarkers to prevent or detect AKI early. The ADQI has assigned the highest research priority to the evaluation of new biomarkers.[25] The aim of our study was to analyze one of the most relevant renal biomarkers (NGAL) in its urinary form, in patients with infections, and thus to verify its diagnostic and prognostic characteristics for AKI.

While analyzing the results of our study, we were not surprised by several findings, due to the inclusion of serious patients from public hospitals, which attend individuals who have many comorbidities, including factors that are associated with AKI. Based on the above, we found a high incidence of AKI (76.3%, n = 29), high incidence and compatible with other studies.[3,26]

Table 2. Statistical Differences Between Quantitative Variables and Measurements of uNGAL.

	Without AKI (n = 9)		With AKI (n = 29)			
	Median	IQR (25%-75%)	Median	IQR (25%-75%)	*P* value	Multivariable analysis
Age (years)	60	45-74.5	75	54.5-82.5	.086	
Temperature (°C)	36	35.4-37.6	36.1	35.2-37.2	.586	
MAP (mmHg)	73	60-92.5	61	50-74.5	.152	
HR (/min)	106	94-121.5	104	84.5-120	.521	
RR (/min)	21	19-22	26	17-31.5	.399	
PaO_2 (mmHg)	92	76-122	78	62.5-113.5	.173	
pH	7.4	7.38-7.45	7.4	7.3-7.42	.234	
Cr at Day 0 (mg/dL)	0.8	0.5-0.9	1.3	0.5-2.1	.058	
Leukocytes (/10^3mm^3)	9200	4620-19 090	12 990	10 200-20 900	.133	
Bilirubin (mg/dL)	1.15	0.2-2	0.64	0.5-1.1	.397	
Hospitalization duration (days)	13	7-18	21	13.5-25	.019	NS
Charlson index	2	1-2.5	4	3-7.5	.002	NS
APACHE II	17	12-18	25	19.5-27.5	<.001	NS
qSOFA	1	0-2	2	1-3	.032	NS
SOFA on Day 1	3	1.5-5	8	4-12	.008	NS
Lactate on Day 1 (mm/L)	1.9	1.7-2.8	1.9	1.3-2.8	.754	
uNGAL on Day 1 (ng/mL)	78.2	32.4-198.5	634.4	215.9-1547.1	<.001	[a]
uNGAL on Day 2 (ng/mL)	55.7	26.3-233.3	468.9	263.9-1384.5	<.001	[a]
uNGAL on Day 3 (ng/mL)	45.3	14.3-208.5	794	216.2-1993.0	<.001	[a]
uNGAL on Day 4 (ng/mL)	66.2	15.4-179.9	597.7	121.7-2017.2	.003	[a]

Note. uNGAL = urinary neutrophil gelatinase-associated lipocalin; AKI = acute kidney injury; IQR = interquartile range; MAP = mean arterial pressure; HR = heart rate; RR = respiratory rate; PaO_2 = partial oxygen pressure; Cr = creatinine; NS = not significant; APACHE II = Acute Physiology and Chronic Health disease Classification System II; qSOFA = quick SOFA; SOFA = Sequential Organ Failure Assessment.
[a]Not included in the model for risk factor.

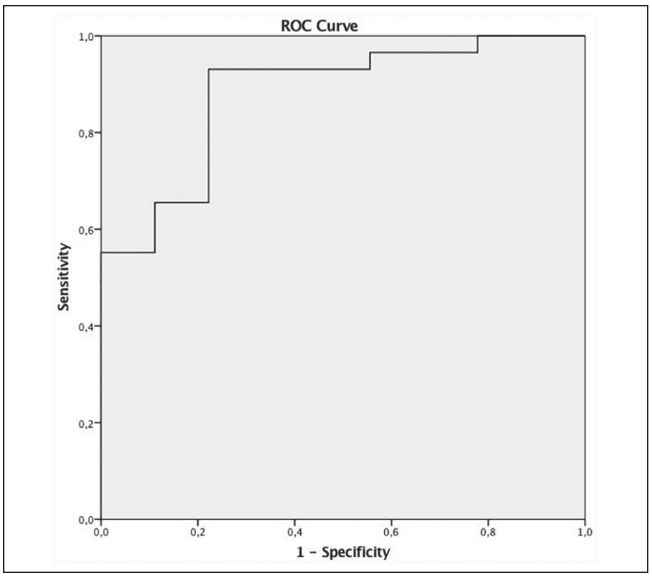

Figure 1. ROC curve: uNGAL performance in detecting AKI.
Note. The KDIGO is gold standard. ROC curve was 0.881. ROC = receiver operating characteristic; uNGAL = urinary neutrophil gelatinase-associated lipocalin; AKI = acute kidney injury; KDIGO = Kidney Disease Improving Global Outcomes.

While analyzing the results of our study, we were not surprised by several findings, due to the inclusion of serious patients from public hospitals which attend individuals who have many comorbidities, including factors that are associated with AKI. Our affirmations are based on the fact that in the AKI group we found a high frequency of systemic arterial hypertension, immunosuppression, peripheral arterial disease and diabetes, as well as high values of prognostic scores in the 2 initial groups, which were analyzed (without AKI and with AKI). As for example, APACHE II had a median of 17 in the group without AKI and a median of 25 in patients who had AKI; the SOFA score on the first day had a median of 3 in the non-AKI group and a median of 8 in the AKI group. Other studies had already clarified the most frequent etiologies of AKI,[3,19,26,27] as found in our findings. The median scores for SOFA, Charlson criteria, qSOFA, and APACHE II were higher in patients with AKI, with statistical significance ($P < .05$).

Our AKI patients also had a higher median of hospitalization, with statistical significance. As has been documented in other studies,[28] it is possible that the extended hospital permanence of AKI patients led to higher costs for the public health system.

While the frequently prescribed drugs such as antimicrobials, especially beta-lactam antibiotics, vancomycin, aminoglycosides, antiviral drugs (acyclovir), and antifungals (primarily nonliposomal amphotericin B) have been reported as causative factors of AKI,[3] we did not find a statistically significant association in our study. We believe that this discrepancy exists because of the small number of patients in our study.

Table 3. uNGAL Values in the KDIGO Classification and Without AKI.

		uNGAL 1		uNGAL 2		uNGAL 3		uNGAL 4	
	N	Median	IQR (25%-75%)	Median	IQR (25%-75%)	Median	IQR (25%-75%)	Median	IQR (25%-75%)
No AKI	9	78.2	32.4-198.5	55.7	26.3-233.3	45.3	14.3-208.5	66.2	15.4-179.9
AKI stage 1	6	213.0	152.8-868.3	259.4	62.0-894.5	158.2	41.7-926.3	63.3	21.1-586.4
AKI stage 2	4	317.1	104.0-1802.4	318.8	117.7-895.6	513.2	133.8-916.2	560.1	145.1-928.9
AKI stage 3	19	937.1	504.4-1700.0	1026.4	368.9-2090.3	961.6	374.1-2377.5	794.6	403.9-4666.4

Note. uNGAL = urinary neutrophil gelatinase-associated lipocalin; KDIGO = Kidney Disease Improving Global Outcomes; AKI = acute kidney injury; IQR = interquartile range.

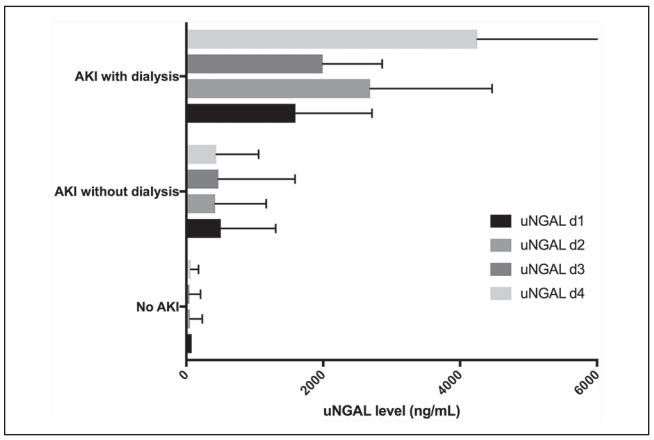

Figure 2. Differences of uNGAL in groups.
Note. AKI with dialysis (N = 6). AKI without dialysis (N = 23). No AKI (N = 9). uNGAL d1= uNGAL on day 1 (ng/mL). uNGAL d2= uNGAL on day 2 (ng/mL). uNGAL d3= uNGAL on day 3 (ng/mL). uNGAL d4= uNGAL on day 4 (ng/mL). uNGAL = urinary neutrophil gelatinase-associated lipocalin; AKI = acute kidney injury.

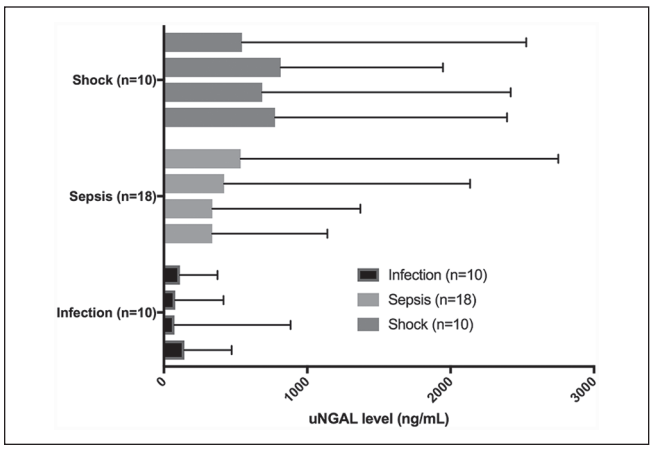

Figure 3. uNGAL (ng/mL) on the 4 days (d1-d4) in infected patients.
Note. uNGAL = urinary neutrophil gelatinase-associated lipocalin.

In our patients, and we observed, through the analysis of the subdivisions regarding the severity of the existing infectious disease, that in all 3 groups the presence of AKI as a marker of organic dysfunction was high: among patients with sepsis (18 cases), 77.7% developed AKI; 90% (n = 9) of septic shock patients developed AKI; and 20% (n = 2) of patients who had no other organ dysfunction (SOFA < 2) progressed to AKI during hospitalization. Moreover, our data of AKI cases were similar to those reported in the literature regarding ICUs with more critical patients.[8]

The 10th ADQI Consensus Conference proposed the utilization of both function and damage biomarkers in combination with traditional markers of renal function to better define and characterize AKI.[21] The biomarkers over the last decade have been evaluated for their capacity to detect kidney "stress" and/or "damage" and to predict the development of AKI. They apply to septic AKI as well. The strong interest in biomarkers relates to the desire to achieve early diagnosis to deliver prevention and early therapy when it may be most effective.[29]

In our study, we identified that the performance of uNGAL in predicting AKI was high during the 4 days analyzed. The cutoff levels of uNGAL that identified patients who would progress to AKI were the following: (d1) >116 ng/mL; (d2) >100 ng/mL, and (d3) 284 ng/mL. Having interest in early diagnosis, we esteem the value d1 (up to 24 hours after clinical screening). These values, especially the first one, are equivalent to values already found in the literature.[12] Other studies have shown an association between the urinary NGAL curve and progression to AKI in patients with severe infections.[7,30] A wide range of cutoff levels of uNGAL have been used in various studies; however, a level of > 150 ng/mL appeared to be the most appropriate, particularly when commercial assays were used.[31]

The AUC-ROC was 0.881 when we analyzed the performance of uNGAL for detecting AKI in patients with infections by using KDIGO criteria. A systematic literature review verifying the role of serum and urinary NGAL in predicting AKI found that AuROC values ranged from 0.54 to 0.98. The considerable variability found in the ROC curve occurred because of the heterogeneity and designs of the studies included.[32]

Regarding the prognostic context, we observed that uNGAL values increased according to the progress of the KDIGO classification (AKI 1, AKI 2, and AKI 3). It is clearly known that the progression in the KDIGO classification

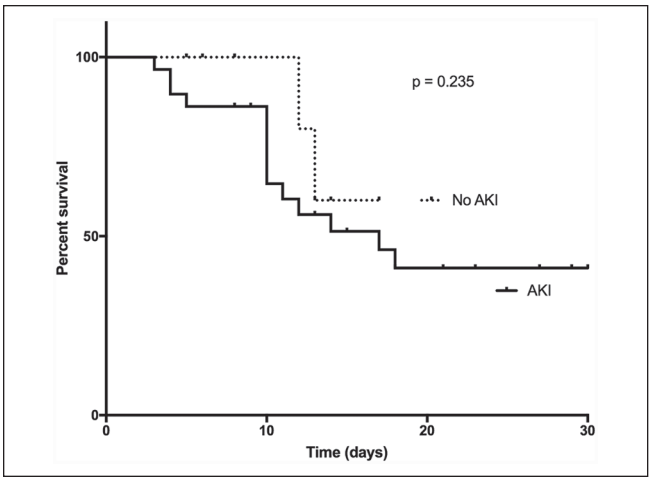

Figure 4. Survival curve between groups.

indicates a worse prognosis.[19] Acknowledging the correlation between the functional loss of renal physiology with the worsening of the KDIGO score, we can try to correlate the uNGAL values, promoting clinical measures that reduce the worsening of renal function.

As to the analysis of uNGAL, regarding the three groups of patients who were screened (infected patients, patients with sepsis, and septic shock), we observed the association of uNGAL peaks with the disease severity: septic shock and sepsis, presenting higher values and statistical significance, demonstrating prognostic characteristics, because we know that the greater number of organ dysfunctions in sepsis indicates more chance of deaths.[17] Here again uNGAL could be used as another tool that stratifies severity in patients with infections.

As of RRT, we noticed in our study an association of urinary biomarker peaks with the need for intervention, even when compared to patients with AKI who did not dialyze. A recent meta-analysis aimed at demonstrating the use of renal biomarkers (serum and urinary) while predicting who will need RRT identified that among the 9 urinary biomarkers evaluated, uNGAL was the most included in studies with an AUC of 0.720 (95% confidence interval [CI]: 0.638-0.803) of diagnostic prediction.[33] An important study published in 2016 used the dosage of a biomarker and its cutoff value as a criterion for patient selection. The purpose of the study was to evaluate the best time to start RRT.[34]

The overall mortality rate, during 30 days of the patient hospitalization, was of 42.1%. There was, however, no statistic difference regarding mortality rate of patients with AKI and those without AKI ($P = .235$). The group without AKI was a small sample, leading to no statistical relevance. According to the classification of infection severity, the mortality found was infected patients without organic dysfunction (20%), patients with sepsis (38%) and septic shock (70%). Our mortality findings were like those reported in other epidemiological studies.[3,35]

Moreover, 83.3% of patients undergoing RRT died. This last rate of mortality in dialysis patients was concerning, possibly due to the severity of the patients who needed RRT or a late timing as to dialysis support (90% of patients started RRT in KDIGO Level 3). Having in mind that the RRT initiation timing has been the subject of extensive investigation over decades, but specifically during the last couple of years, 2 randomized studies/trials had conflicting results. One of the studies included the measurement of serum NGAL during patient recruitment.[20,34,36]

The higher median uNGAL on Days 1, 3, and 4 were also associated with mortality in 30 days (d1, $P = .039$; d3, $P = .005$; d4, $P = .005$). Previous studies associated higher uNGAL values with mortality. One important meta-analysis concluded that NGAL not only is an effective predictor of AKI in the process of sepsis but also has a potential predictive value for RRT and mortality. However, future trials are needed to clarify this controversial issue.[37]

AKI biomarkers have been introduced and validated in many studies during the past 10 years; however, there is still cause for extensive debate regarding the efficacy of its application at the bedside. A point of inquiry in the medical literature is whether the increase in serum or urinary biomarker values is not associated with the development of AKI in all patients, or rather have a correlation with the patient's severity and systemic inflammatory curve.[38] In our study, we had 6 patients who were classified in the "infection without organic dysfunction" group but developed AKI. That is, of the 28 patients who presented AKI, 21.4% (n = 6) were in a group that did not have organic dysfunction. We believe that there was some discriminatory potential from uNGAL in our work in predicting patients who developed AKI.

We must remember that in the use of a tool that acts in the diagnosis, the cost-benefit relation must be analyzed. A cost-effectiveness study of the use of serum or urinary NGAL in the early diagnosis of AKI in cardiovascular surgical patients concluded that the biomarker has economic advantages when used.[39]

Limitations

This was a double-center study, and our findings may not be applicable to centers with differing profiles of patients with infections. We also understand that results different from our results may occur regarding uNGAL assessments and that the use of other biomarkers of kidney injury may be more accurate in the stratification of patients who are at risk for AKI. Moreover, it is difficult to compare studies using different commercial NGAL assays, and the lack of concordance among such assays has been clearly demonstrated. Different commercial assays use different monoclonal and polyclonal antibody combinations, have different epitope specificities, and detect different proportions of the various molecular forms of uNGAL. A major systematic review published within the past decade identified substantial diversity in the

methodologies used when NGAL values are employed for AKI diagnosis and for estimating mortality rates.[32]

Two other important limitations of our work were that the urinary NGAL values were not adjusted for urinary creatinine and the water balance was not evaluated. These may have contributed to the variability of the results of uNGAL.

Conclusions

The uNGAL had an association in its values with the diagnosis and prognosis of patients with severe infections and AKI. We suggest that studies with a greater number of patients could better establish the cutoff values of uNGAL and/or serum NGAL in the identification of infected patients who are at a high risk of developing AKI, thereby stimulating possible actions to prevent disease progression.

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List of Abbreviations

AKI, acute kidney injury; ICU, intensive care unit; KDIGO, Kidney Disease Improving Global Outcomes; RRT, renal replacement therapy; SCr, serum creatinine; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

Ethics Approval and Consent to Participate

The study was approved by the Research Ethics Committee of Hospital de Clínicas de Curitiba-PR.

Consent for Publication

Informed consent was obtained from all patients or their legal representatives.

Availability of Data and Material

The data supporting our findings are available on request from the corresponding author.

Author Contributions

B.C.G. and F.F.T. conceived of this study and participated in the design of the study. F.F.T. performed the statistical analysis, helped in the interpretation of data, and drafted the manuscript. B.C.G. and J.M.S.Jr performed the collection of data. J.M.S.Jr helped in revising the draft of the manuscript. B.C.G., F.F.T., and J.M.S.Jr helped in the final revision of and writing the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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ORCID iD

Brenno Cardoso Gomes https://orcid.org/0000-0002-3065-1512

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