Role of Tissue Inhibitor of Metalloproteinases-2 and Insulin Like Growth Factor Binding Protein 7 for Early Recognition of Acute Kidney Injury in Critically Ill COVID-19 Patients

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Abstract

Background: A high proportion of critically ill patients with COVID-19 develop acute kidney injury (AKI) and die. Early recognition of subclinical AKI could contribute to AKI prevention. Therefore, this study was aimed at exploring the role of the urinary biomarkers NGAL and [TIMP-2]•[IGFBP7] for early detection of AKI in this population.

Methods: This prospective, longitudinal cohort study included critically ill COVID-19 patients without AKI at study entry. Urine samples were collected on admission to critical care areas for determination of NGAL and [TIMP-2]•[IGFBP7] concentrations. Demographic information, comorbidities, clinical and laboratory data were recorded. The study outcomes were development of AKI and mortality during hospitalization. Comparisons of individuals who developed AKI during hospitalization vs. those without AKI were made using chi-squared test for categorical variables and Mann-Whitney U for continuous variables. Urinary biomarkers and their cutoff values were selected based on the highest sensitivity, specificity and area under the receiver-operating characteristics curve with 95% confidence intervals for prediction of AKI. Selected biomarkers and cutoffs were used in the Kaplan-Meier survival analyses for the time to AKI. Logistic regression analysis was used to identify the association between relevant covariates with AKI and mortality. For all analyses, two-sided P values ≤ 0.05 were considered statistically significant.

Results: Of the 51 individuals studied, 25 developed AKI during hospitalization (49%). The risk factors for AKI were male gender (HR=7.57, 95% CI: 1.28-44.8; $p=0.026$) and [TIMP-2]•[IGFBP7] ≥ 0.2 (ng/ml)²/1000 (HR=7.23, 95% CI: 0.99-52.4; $p=0.050$). Mortality during hospitalization was significantly higher in the group with AKI than in the group without AKI ($p=0.004$). Persistent AKI was a risk factor for mortality (HR=7.42, 95% CI: 1.04-53.04; $p=0.046$).

Conclusions: The combination of [TIMP-2]•[IGFBP7], together with clinical information, were useful for identification of subclinical AKI in critically ill COVID-19 patients. The role of additional biomarkers and their possible combinations for detection of AKI in critically ill COVID-19 patients remains to be explored in large clinical trials.

Background

The clinical spectrum resulting from infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), ranges from an asymptomatic response or development of a mild upper respiratory tract infection to critical coronavirus disease 2019 (COVID-19) [1]. The rates of acute kidney injury (AKI) in patients with severe COVID-19 are extremely variable, but evidence suggests that it likely affects > 20% of hospitalized patients and > 50% of patients in the intensive care unit (ICU) [2]. Traditionally, AKI diagnosis is based on changes in kidney function such as increased serum creatinine (sCr), which is a low sensitivity method because nearly 50% of glomerular filtration rate (GFR) must be lost before a change in sCr is detectable; and decreased urine output, which lacks specificity since it may be triggered by hypovolemia, without direct damage to the kidney [3]. Recognition of AKI is unacceptably delayed in up to 43% of hospitalized patients [4], leading to loss of therapeutic windows. The elevation of urinary biomarkers of kidney stress in the absence of changes in sCr and urine output has been considered as subclinical AKI, a term identifying those patients at high risk for AKI [5]. These patients are likely to be the ones who would benefit from the use of biomarkers and early interventions. In this context, the tissue inhibitor of metalloproteinases-2 (TIMP-2) and the insulin-like growth factor binding protein 7 (IGFBP7)

have been identified as possible AKI biomarkers, given that both are released following ischemic or inflammatory processes in the kidney, resulting in G1 cell cycle arrest for a short period [6, 7, 8]. Another biomarker of early AKI is the urinary neutrophil gelatinase-associated lipocalin (NGAL), as intrarenal concentration of this protein is abruptly up-regulated soon after ischemic or nephrotoxic kidney injury [9]. In view of the need to identify early signs of kidney involvement, this study was aimed at exploring the role of the urinary biomarkers NGAL, TIMP-2 and IGFBP7 for early detection of AKI in critically ill patients with COVID-19.

Methods

Study Population

This prospective, longitudinal cohort study was conducted at the National Institute of Respiratory Diseases (INER), the largest third-level institution designated by the Mexican Government for COVID-19 care. The Institutional Review Board approved the study (Approval No C26-20) and written informed consent was obtained from all participants. We included individuals admitted to the ICU with diagnosis of severe pneumonia caused by SARS-CoV-2, who were 18 years of age or older; without AKI when urine sample was collected; with no history of chronic kidney disease (CKD) as indicated by interrogation of patients about CKD medical history and by an estimated glomerular filtration rate (eGFR) greater than 60 ml/min/1.73m² using the CKD-EPI equation [10]. SARS-CoV-2 severe pneumonia was defined by clinical data of respiratory distress, bilateral alveolar opacities in 2 or more lobes, a ratio of partial arterial oxygen pressure/inspired oxygen fraction (PaO₂/FiO₂) < 300 mm Hg and a positive result for SARS-CoV-2-real-time reverse transcription–polymerase chain reaction (rRT-PCR) assay in nasopharyngeal swab [11]. The primary outcome was the development of AKI during hospitalization. The secondary outcome was mortality during hospitalization in the group with AKI and the group without AKI. Recorded variables included demographic and anthropometric variables, symptoms, comorbidities, treatments, critical care variables, blood chemistry, blood count, starting and termination dates of invasive mechanical ventilation (IMV), days in hospital, initial mechanical-ventilator settings, use of vasoactive drugs and outcomes. Pregnant women were not included in the study. Patients with incomplete clinical records were excluded.

Definition of acute kidney injury

AKI staging was based on serum creatinine (sCr) levels. The urine output criterion was not used for diagnosis of AKI since nursing records were out of reach, in COVID-19 areas. The baseline sCr level was defined as the minimum inpatient value during the first 7 days of admission [12]. Diagnosis of AKI was based on the Kidney Disease Improving Global Outcomes (KDIGO) criteria [13]. AKI stage 1 corresponded to an increase in sCr by ≥ 0.3 mg/dl within 48 hours or increase in sCr 1.5 to 1.9 times baseline within the prior 7 days; AKI stage 2 corresponded to an increase in sCr of 2.0-2.9 times baseline; and AKI stage 3 corresponded to an increase in sCr of ≥ 3 times baseline or initiation of renal replacement therapy. Persistent AKI was defined by the continuance of AKI by serum creatinine or urine output criteria (as defined by KDIGO) beyond 48 h from AKI onset. Transient AKI was defined by complete reversal of AKI by KDIGO criteria within 48 h of AKI onset [14].

Biomarker determinations

Urine samples were collected on admission to critical care areas (day 1). Urine was frozen at -80 C within the first 30 minutes after sample collection. Urinary concentrations of TIMP-2 and IGFBP7 were determined using commercially available ELISA kits (Human TIMP-2 Quantikine ELISA Kit, R&D, Minneapolis, Minnesota; Human

IGFBP7 ELISA Kit, Abcam, Cambridge, UK) following manual instructions. ELISA plates were read at O.D. of 450 and calculations were done according to the signal given by the standard curve of each kit. NGAL determinations were done using the NGAL kit (Abbott, Chicago, Illinois) according to the manual instructions and using the Abbott™ ARCHITECT™ Analyzer.

Statistical Analysis

We performed descriptive statistics including means and standard deviations for normally distributed continuous variables, medians and interquartile ranges for non-parametric distributions, and proportions for categorical variables. Comparisons of individuals who developed AKI during hospitalization vs. those without AKI were made using chi-squared test for categorical variables and Mann-Whitney U for continuous variables.

For each biomarker, the area under the receiver-operating characteristics curve (AUC) with 95% confidence intervals was calculated, as well as the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy at 3 different cutoff values using urine samples collected upon hospital admission. We considered that the prevalence of AKI for patients with SARS-CoV-2 infection in the ICU was 40% [15]. Cutoffs for each biomarker were selected based on the highest AUC, specificity and accuracy for prediction of AKI. Combinations of the top biomarkers were also explored. When combinations had no significant added value, individual biomarkers were preferred. For all analyses, two-sided P values ≤ 0.05 were considered statistically significant. The selected biomarkers and cutoff values were used in the Kaplan-Meier survival analyses for the time to AKI.

Logistic regression analysis was used to identify the association between relevant covariates with AKI and mortality. We obtained age-stratified estimates considering 60 years and older as vulnerable population. Variables were entered into the models when the alpha level of risk factor was < 0.20 in the univariate analysis. Age and gender were entered into the models regardless of the alpha level. All statistical tests were two-sided, and two-sided P values ≤ 0.05 were considered statistically significant. The analysis was conducted using RStudio 1.4.1717.

Results

Characteristics of study population

During the period between May and August 2020, a total of 420 individuals were admitted to critical areas of the INER. Of those, 69 were negative for SARS-CoV-2 infection; in 44 the infection could not be confirmed; 60 remained in the emergency room due to hospital saturation and 20 died there. Informed consent could not be obtained for 196 patients. We thus included the 51 patients who provided informed consent for participating in the study (Fig. 1). Of those, 30 were male (58.8%); the median age was 53 years (IQR, 40–61); 14 had hypertension (27.5%); 16 had diabetes (31.4%); and 21 were obese 41.2% (Table 1). Of the 51 individuals studied, 25 developed AKI during hospitalization (the AKI group, 49.0%) and 26 did not develop AKI (the non-AKI group, 51.0%). Eleven individuals had AKI stage 1 (21.5%); 8 had AKI stage 2 (15.6%); and 6 had AKI stage 3 (11.7%). The AKI group was older than the non-AKI-group (57 years, IQR: 47–65, vs. 49 years, IQR: 37–56; $p = 0.029$). Hypertension was more frequent in the AKI group than in the non-AKI group (44.0% vs. 11.5%; $p = 0.009$). A lower proportion of patients in the AKI group was receiving lopinavir/ritonavir (8.0% vs. 38.5%; $p = 0.010$) compared to the non-AKI group. Serum creatinine (0.64 mg/dl, IQR: 0.53–0.80, vs. 0.56 mg/dl, IQR: 0.43–0.70; $p = 0.039$), as

well as blood urea nitrogen (32.0 mg/dl, IQR: 27–45, vs. 13.5 mg/dl, IQR: 11–21; $p = 0.001$) were higher in the AKI group than in the non-AKI group; while the estimated glomerular filtration rate (eGFR) was significantly lower in the AKI group (107.02 ml/min, IQR: 92.5–114.9, vs. 113.3 ml/min, IQR: 108.7–125.1; $p = 0.026$). The AKI group had higher levels of creatine phosphokinase (175.0 U/L, IQR: 97–992, vs. 58 U/L, IQR: 31–354; $p = 0.004$); C-reactive protein (25.2 mg/dL, IQR: 13.6–31.9, vs. 13.2 mg/dL, IQR: 10.1–17.7; $p = 0.030$); procalcitonin (0.60 ng/ml, IQR: 0.17–1.08, vs. 0.19 ng/ml, IQR: 0.08–0.52; $p = 0.043$); and troponin (12.4 pg/ml, IQR: 3.8–40.6, vs. 4.2 pg/ml, IQR: 2.1–9.0); $p = 0.039$) than the non-AKI group. The AKI group also had significantly higher levels of urinary biomarkers like NGAL (50.2 ng/ml, IQR: 36.4–112.1, vs. 32.9 ng/ml, IQR: 13.9–44.7; $p = 0.015$); IGFBP7 (22.05 ng/ml, IQR: 9.93–29.08, vs. 11.57 ng/ml, IQR: 7.04–17.73; $p = 0.040$); and $[TIMP-2] \cdot [IGFBP7]$ ($0.113 \text{ (ng/ml)}^2/1000$, IQR: 0.055–0.270, vs. $0.059 \text{ (ng/ml)}^2/1000$, IQR: 0.027–0.127; $p = 0.026$) than the non-AKI group. As expected, mortality was higher in the group with AKI than in the non-AKI group (36.0% vs. 3.8%; $p = 0.004$).

Table 1
Baseline characteristics of study population.

Variable	Overall (n = 51)	AKI (n = 25)	Non-AKI (n = 26)	p value
Age, years *	53 (40–61)	57 (47–65)	49 (37–56)	0.029
Male [n (%)]	30 (58.8)	18 (72)	12 (46.1)	0.061
BMI, kg/m ² *	29.3 (25.9–31.6)	29.4 (26.7–34.1)	29.1 (25.9–30.3)	0.522
Comorbidities				
Obesity [n (%)]	21 (41.2)	11 (44.0)	10 (38.5)	0.688
Diabetes [n (%)]	16 (31.4)	7 (28.0)	9 (34.6)	0.611
Hypertension [n (%)]	14 (27.5)	11 (44.0)	3 (11.5)	0.009
Two or more comorbidities [n (%)]	21 (41.2)	12 (48.0)	9 (34.6)	0.332
Critical care variables				
IMV [n (%)]	32 (62.7)	18 (72.0)	14 (53.8)	0.180
PaO ₂ /FiO ₂ ratio, mmHg *	141 (108–187)	140 (120-174.5)	148 (103-198.5)	0.733
PEEP, cm H ₂ O *	10 (9.5–14)	10 (8–12)	12 (10–14)	0.125
pH *	7.4 (7.3–7.4)	7.3 (7.3–7.4)	7.4 (7.3–7.4)	0.556
pCO ₂ , mmHg *	38.0 (32.3–51.9)	46.3 (33.0-52.2)	35.9 (33-50.9)	0.378
SOFA score, points *	4 (2–6)	4 (3–6)	3 (2–6)	0.076
Vasoactive drugs [n (%)]	16 (31.4)	8 (32.0)	8 (30.8)	0.925
Inotropic drug [n (%)]	2 (3.9)	0 (0.0)	2 (7.7)	0.157
Treatment				
Systemic steroids [n (%)]	25 (49)	14 (56.0)	11 (42.3)	0.328
Tocilizumab [n (%)]	25 (49)	11 (44.0)	14 (53.8)	0.355
Hydroxychloroquine [n (%)]	7 (13.7)	4 (16.0)	3 (11.5)	0.643
Lopinavir/Ritonavir [n (%)]	12 (23.5)	2 (8.0)	10 (38.5)	0.010
Nephrotoxic drugs [n (%)]	1 (2)	0 (0.0)	1 (3.8)	0.322
Renal function indicators				

AKI, acute kidney injury; BMI, body mass Index; IMV, invasive mechanical ventilation; PaO₂/FiO₂, partial arterial oxygen pressure/inspired oxygen fraction; pCO₂, partial pressure of carbon dioxide; PEEP, positive end-expiratory pressure; SOFA, sequential organ failure assessment; eGFR, estimated glomerular filtration rate; Systemic steroids: dexamethasone, methylprednisolone; prednisone; NGAL, neutrophil gelatinase-associated lipocalin; TIMP2, tissue inhibitor of metalloproteinases 2; IGFBP7, insulin like growth factor binding protein 7.

*Data expressed as medians (interquartile ranges).

Variable	Overall (n = 51)	AKI (n = 25)	Non-AKI (n = 26)	p value
Serum creatinine, mg/dl, Day 1 *	0.6 (0.5–0.7)	0.6 (0.5–0.8)	0.6 (0.4–0.7)	0.039
eGFR, ml/min, Day 1*	112.3 (98.4-121.4)	107.0 (92.5-114.9)	113.3 (108.7-125.1)	0.026
Laboratories				
Hemoglobin, g/dL, Day 1*	13.3 (12.6–14.9)	13.1 (12.5–15.0)	13.4 (12.8–14.4)	0.850
Leucocytes, 10x ³ mm ³ , Day 1*	8.9 (6.3–13.4)	9.4 (7.8–13.4)	8.3 (5.8–11.6)	0.239
Lymphocytes, 10x ³ mm ³ , Day 1*	0.8 (0.6-1.0)	0.7 (0.5-1.0)	0.85 (0.6–1.1)	0.219
Platelets, 10x ³ mm ³ , Day 1*	272 (219–329)	283 (228–363)	259 (219–308)	0.323
Blood urea nitrogen, mg/dL, Day 1 *	22 (13–35)	32 (27–45)	13.5 (11–21)	0.001
Lactate dehydrogenase, U/ml, Day 1 *	387 (299–557)	421 (327–557)	364.5 (249–541)	0.118
Total bilirrubines, mg/dL, Day 1 *	0.5 (0.4–0.6)	0.5 (0.4–0.6)	0.4 (0.4–0.8)	0.651
Creatine phosphokinase, U/L, Day 1 *	140 (39–443)	175 (97–992)	58 (31–354)	0.004
D-dimer, µg/ml, Day 1 *	0.9 (0.4–2.5)	1.1 (0.5–3.7)	0.7 (0.4–1.2)	0.057
C-reactive protein, mg/dL, Day 1 *	16.5 (10.3–27.6)	25.2 (13.6–31.9)	13.2 (10.1–17.7)	0.030
Fibrinogen, mg/dL, Day 1 *	733.5 (580.2-821.2)	750 (613.5–805)	685 (587–786)	0.424
Procalcitonin, ng/ml, Day 1 *	0.4 (0.1–0.9)	0.6 (0.2–1.1)	0.1 (0.9 – 0.5)	0.043
Troponin, pg/ml, Day 1 *	5.6 (3.3–37)	12.4 (3.8–40.6)	4.2 (2.1-9.0)	0.039
Ferritin, ng/ml, Day 1 *	745.4 (358.3-1883.4)	831.3 (468.2–2512)	636.4 (337.1-1008.7)	0.257
Urinary Biomarkers				
NGAL, ng/ml, Day 1 *	39.3 (19.2–98.5)	50.2 (36.4-112.1)	32.9 (13.9–44.7)	0.015
TIMP-2, ng/ml, Day 1 *	5.5 (3.0–9.0)	6.3 (4.2–9.1)	5.1 (2.9–7.6)	0.356
IGFBP7, ng/ml/1000, Day 1*	13.4 (8.2–24.1)	22.1 (9.9–29.0)	11.6 (7.0-17.7)	0.040
[TIMP-2]•[IGFBP7], (ng/ml) ² /1000, Day 1*	0.9 (0.5 – 0.2)	0.1 (0.1–0.3)	0.6 (0.1–0.1)	0.026
Outcomes				
AKI, acute kidney injury; BMI, body mass Index; IMV, invasive mechanical ventilation; PaO ₂ /FiO ₂ , partial arterial oxygen pressure/inspired oxygen fraction; pCO ₂ , partial pressure of carbon dioxide; PEEP, positive end-expiratory pressure; SOFA, sequential organ failure assessment; eGFR, estimated glomerular filtration rate; Systemic steroids: dexamethasone, methylprednisolone; prednisone; NGAL, neutrophil gelatinase-associated lipocalin; TIMP2, tissue inhibitor of metalloproteinases 2; IGFBP7, insulin like growth factor binding protein 7.				
*Data expressed as medians (interquartile ranges).				

Variable	Overall (n = 51)	AKI (n = 25)	Non-AKI (n = 26)	p value
Days in hospital	16 (12–27)	20 (14–27)	14.5 (12–26)	0.304
Days on IMV	13 (10–22.2)	15 (12.5–22)	11 (8.5–21.5)	0.235
Days with symptoms before IMV	10 (6–14)	9 (5.5–12)	11.5 (8–14)	0.178
Mortality [n (%)]	10 (19.6)	9 (36.0)	1 (3.8)	0.004
AKI, acute kidney injury; BMI, body mass Index; IMV, invasive mechanical ventilation; PaO ₂ /FiO ₂ , partial arterial oxygen pressure/inspired oxygen fraction; pCO ₂ , partial pressure of carbon dioxide; PEEP, positive end-expiratory pressure; SOFA, sequential organ failure assessment; eGFR, estimated glomerular filtration rate; Systemic steroids: dexamethasone, methylprednisolone; prednisone; NGAL, neutrophil gelatinase-associated lipocalin; TIMP2, tissue inhibitor of metalloproteinases 2; IGFBP7, insulin like growth factor binding protein 7.				
*Data expressed as medians (interquartile ranges).				

Performance of biomarkers as AKI predictors

Based on the highest AUC, specificity and accuracy values, the biomarker with best performance for AKI prediction during the whole hospitalization period was NGAL at a cutoff of 45 ng/ml. Considering that most patients developed AKI during the first 7 days at the hospital, we also determined the performance of biomarkers for AKI prediction on day 7 (Table 2). The performance of NGAL was significantly better on day 7 than during the whole hospitalization period (AUC = 0.706 vs. AUC = 0.771; p = 0.001). The combination of [TIMP-2]•[IGFBP7] at a cutoff of 0.2 (ng/ml)²/1000 were the second best AKI predictors, and the performance of these biomarkers during the whole hospitalization was similar to that of day 7 (AUC = 0.682 vs. AUC = 0.671; p = 0.632).

Table 2

Performance of urinary biomarkers for prediction of acute kidney injury in critically ill COVID-19 patients.

Biomarker	AUC	95% CI	p	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Prediction of AKI during the whole period of hospitalization									
N-Gal (ng/ml)	0.706	0.559-0.854	0.015	40.0	59.1	61.5	50.6	69.3	60.5
				45.0	54.5	76.9	61.1	71.7	67.9
				50.0	50	76.9	59.1	69.7	66.1
[TIMP-2]•[IGFBP7] ((ng/ml) ² /1000)	0.682	0.535-0.829	0.026	0.1	56.0	69.2	54.8	70.2	63.9
				0.2	40.0	88.4	69.8	68.8	69.1
				0.3	24.0	92.3	67.5	64.5	64.9
Prediction of AKI on day 7 of hospitalization									
N-Gal (ng/ml)	0.771	0.632-0.910	0.002	40.0	70.5	64.5	57.0	76.6	66.9
				45.0	64.7	77.4	65.6	76.6	72.3
				50.0	64.7	80.6	69.0	77.4	74.2
[TIMP-2]•[IGFBP7] ((ng/ml) ² /1000)	0.671	0.515-0.827	0.041	0.1	60.0	67.7	55.3	71.7	64.6
				0.2	45.0	87.1	69.9	70.3	70.2
				0.3	25.0	90.3	63.2	64.3	64.1

AKI, acute kidney injury; AUC, area under the receiver-operating characteristics curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; NGAL; neutrophil gelatinase-associated lipocalin; TIMP-2, tissue inhibitor of metalloproteinases-2; IGFBP7, insulin like growth factor binding protein 7.

Time to AKI was significantly shorter in individuals with higher values of urinary NGAL and [TIMP-2]•[IGFBP7]

As NGAL 45 ng/ml had the highest specificity and accuracy values, we selected this cutoff value for the survival analysis of the time to AKI during hospitalization. Individuals with NGAL $\geq$ 45 ng/ml were compared vs. those with $<$ 45 ng/ml. Urinary NGAL could not be measured in 3 patients. The time to AKI was significantly shorter in individuals with NGAL $\geq$ 45 ng/ml than in those with $<$ 45 ng/ml; $p = 0.028$ (Fig. 2). Similarly, [TIMP-2]•[IGFBP7] 0.2 (ng/ml)²/1000 had the highest specificity and accuracy, so this cutoff was selected for the survival analysis of the time to AKI during hospitalization. We found that the time to AKI was significantly shorter in individuals with [TIMP-2]•[IGFBP7] $\geq$ 0.2 ng/ml than in those with $<$ 0.2 (ng/ml)²/1000; $p = 0.017$ (Fig. 3).

Elevated values of urinary [TIMP-2]•[IGFBP7] were risk factors for AKI

The univariate analysis indicated that patients with AKI were older (hazard ratio, HR = 0.94, 95% CI: 0.90–0.99; $p = 0.034$); had a higher frequency of hypertension (HR = 6.02, 95% CI: 1.42–25.40; $p = 0.014$); had levels of [TIMP-

2]•[IGFBP7] ≥ 0.2 (ng/ml)²/1000 (HR = 5.11, 95% CI:1.20-21.67; p = 0.027) and NGAL ≥ 45 ng/ml (HR = 4.00, 95% CI:1.15–13.81; p = 0.028). After adjusting for possible confounding variables, the multivariate analysis indicated that the risk factors for AKI during the hospitalization period were male gender (HR = 7.57, 95% CI:1.28–44.8; p = 0.026) and [TIMP-2]•[IGFBP7] ≥ 0.2 (ng/ml)²/1000 (HR = 7.23, 95% CI: 0.99–52.4; p = 0.050) (Table 3). At day 7 of hospitalization, after adjusting by age and sex, we found that only [TIMP-2]•[IGFBP7] ≥ 0.2 (ng/ml)²/1000 remained associated with risk for AKI (HR = 5.91, 95% CI:1.06–32.7; p = 0.042).

Table 3
Risk factors for acute kidney injury in critically ill COVID-19 patients.

Variables	Unadjusted HR (95% CI)	p value	Adjusted HR (95% CI)	p value
Age	0.94 (0.90–0.99)	0.034	0.95 (0.89–1.01)	0.144
Male	3.0 (0.93–9.61)	0.065	7.57 (1.28–44.8)	0.026
Hypertension	6.02 (1.42–25.40)	0.014	5.61 (0.84–37.22)	0.074
TIMP2*IGFBP7 > 2.0 (ng/ml) ² /1000	5.11 (1.20-21.67)	0.027	7.23 (0.99–52.4)	0.050
NGAL > 45	4.00 (1.15–13.81)	0.028	1.45 (0.30–6.94)	0.637
CPK	1.00 (0.99-1.00)	0.192	-	-
Procalcitonine > 0.25	2.47 (0.79–7.75)	0.119	0.90 (.204.0)	0.894
Troponine	0.99 (0.98-1.00)	0.274	-	-
HR, hazard ratio; CI, confidence interval; NGAL; neutrophil gelatinase-associated lipocalin; TIMP2, tissue inhibitor of metalloproteinases 2; IGFBP7, insulin like growth factor binding protein 7; CPK, creatine phosphokinase.				
Variables were entered into the model when the alpha level of risk factor was less than 0.15. Age and gender were added into the model regardless of the alpha level.				

Mortality was higher in individuals with AKI

We constructed a Kaplan-Meier curve for mortality comparing the group of 25 patients who developed AKI during follow-up, with the group of 26 individuals without AKI. The mortality of individuals who developed AKI at any time during hospitalization was significantly higher than in those who never had AKI, p = 0.019 (Fig. 4).

Of the 25 individuals with AKI, 13 had transient AKI (25.5 %) and 12 had persistent AKI (23.5%). The final CKD-EPI was similar in individuals with persistent AKI (79.5 ml/min/1.73m², IQR: 17–111) and in those with transient AKI (93 ml/min/1.73m², IQR: 88–104; p = 0.53). Logistic regression analysis was used to identify the association between relevant covariates with mortality. The univariate analysis indicated that mortality was more frequent in patients who were 60 years of age or older (HR = 5.33, 95% CI:1.23–23.09; p = 0.025); and had persistent AKI (HR = 10.83, 95% CI:1.67–69.91; p = 0.012). After adjusting for possible confounding variables, only persistent AKI remained associated with mortality (HR = 7.42, 95% CI:1.04–53.04; p = 0.046) (Table 4).

Table 4
Risk Factors for Mortality in critically ill COVID-19 patients.

Variables	Unadjusted HR (95% CI)	p value	Adjusted HR (95% CI)	p value
Age 60, years	5.33 (1.23–23.09)	0.025	3.88 (0.78–19.24)	0.097
Male	3.45 (0.65–18.29)	0.145	2.65 (0.43–16.39)	0.293
Transient AKI	4.33 (0.62–30.24)	0.139	2.47 (0.37–16.38)	0.347
Persistent AKI	10.83 (1.67–69.91)	0.012	7.42 (1.04–53.04)	0.046
AKI, acute kidney injury; HR, hazard rate. Variables were entered into the model when the alpha level of risk factor was less than 0.15. Age and gender were added into the model regardless of the alpha level.				

Discussion

On hospital admission, a large fraction of patients had subclinical signs of kidney dysfunctions that not yet constituted AKI. During subsequent days, AKI became a common complication in our patients, affecting 49% during hospitalization. This frequency was similar to that observed in previous studies, reporting AKI in 50% of the patients with COVID-19 at the ICU [2].

We found that $[TIMP-2] \cdot [IGFBP7] \geq 0.2 \text{ (ng/ml)}^2/1000$ was a risk factor for AKI. In addition, the survival analysis indicated that time to AKI was significantly shorter in individuals with higher $[TIMP2] \cdot [IGFBP7]$. To our knowledge, no large studies have examined the performance of biomarkers for prediction of AKI onset in critically ill patients with COVID-19, but a small study reported that patients with COVID-19-associated AKI and high levels of $[TIMP-2] \cdot [IGFBP7]$ were more likely to progress to renal replacement therapy than those with AKI but with low $[TIMP-2] \cdot [IGFBP7]$ [16]. Our findings are in line with previous reports, describing elevated levels of $[TIMP-2] \cdot [IGFBP7]$ as predictors of adverse outcomes in various clinical conditions, e.g. death, dialysis or progression to severe AKI in patients with septic shock [17]; AKI in patients after major surgery [18]; imminent risk of AKI in critically ill patients [7], and AKI in platinum-treated patients at the ICU [19]. The mechanism proposed is that after initial damage, IGFBP7 and TIMP-2 are expressed in tubular cells. IGFBP7 directly increases the expression of p53 and p21, and TIMP-2 stimulates p27 expression, leading to transitory G1 cell cycle arrest, preventing division of damaged cells [5]. Thus, since G1 cell cycle arrest is a common response to tubular damage, these biomarkers may better reflect damage regardless of etiology.

The combination of $[TIMP-2] \cdot [IGFBP7]$ had the best performance for AKI prediction at values above $0.2 \text{ (ng/ml)}^2/1000$. This cutoff was based on overall behavior of the biomarkers in the patients studied here. However, different cutoffs for these biomarkers have been reported in other studies, so specific groups of patients may require identification of optimal cutoff values, based on their respective values of AUC, sensitivity, specificity, PPV, NPV and accuracy. Cutoff values may be affected by the severity of AKI. That is, higher cutoffs may be found in patients with AKI stages 2 and 3; and lower cutoffs may be found in patients with AKI stage 1 or subclinical AKI. Moreover, AKI is a complex syndrome, involving a series of complex cellular and molecular pathways, and the different cutoffs may reflect mechanistic differences between various etiologies of AKI [5]. The pathophysiologic mechanisms of AKI in COVID-19 are thought to be multifactorial including systemic immune and inflammatory responses induced by viral infection, systemic tissue hypoxia, reduced renal perfusion, endothelial damage and direct epithelial infection with SARS- CoV-2 [20].

In our cohort, the time to AKI was significantly shorter in individuals with NGAL ≥ 45 ng/ml than in those with < 45 ng/ml, but NGAL was not a risk factor for AKI during hospitalization. The fact that performance of NGAL was significantly better on day 7 than during the whole hospitalization period, suggests that NGAL has a narrow predictive time window for AKI. In addition, NGAL has proved less discriminating in the development of septic-associated or adult cardiac-surgery-associated AKI than in other types of AKI, possibly because neutrophils themselves may be a source of NGAL in the setting of systemic inflammation [21].

Contrary to our findings, a recent cohort study found that urinary NGAL > 150 ng/ml predicted diagnosis, duration, and severity of AKI and acute tubular injury, as well as hospital stay, dialysis, shock, and death in patients with acute COVID-19 [22]. Contrasting results may be explained by the fact that some patients in that study probably had AKI when urinary samples were collected, while we only included patients without AKI at the time of urine sample collection. Therefore, the median value of NGAL in the AKI group (50.2 ng/ml) and the selected cutoff (45 ng/ml), were far below in our patients since they had subclinical AKI. In addition, it is unclear if a higher proportion of their patients had AKI stage 2 and stage 3, while most of our patients developed AKI stage 1 on subsequent days. This is relevant because that study also reported a correlation between urinary NGAL levels and AKI severity. In another recent study, NGAL was also found as an independent risk factor for AKI in patients with COVID-19, but that study also included some patients who already had AKI when urine samples were collected [23]. Thus, we suggest that in patients with COVID-19, higher NGAL cutoff values seem to be useful in predicting AKI progression but not AKI onset. In contrast with our findings, urinary NGAL but not [TIMP-2]•[IGFBP7], independently predicted AKI in a cohort of decompensated cirrhotic patients, suggesting that different biomarkers should be used in different patient groups [24].

The survival analysis indicated that mortality was more frequent in patients who developed AKI during hospitalization, and mortality was attributed to persistent AKI because it was a risk factor for mortality. The concept that time should also be considered in the description of AKI and not only severity, was demonstrated in a study reporting that duration of AKI following surgery was independently associated with hospital mortality after adjusting for severity of illness [25]. Transient AKI may reflect a temporary reduction in renal function without structural damage, whereas persistent AKI would reflect structural tubular damage [26]. Based on these observations, persistent AKI has become a relevant endpoint in subsequent studies, and it has consistently been associated with mortality [27].

The use of biomarkers has some limitations, as it should be considered that their value for prediction of AKI is limited to patients who are critically ill. When used in patients who are low risk, the false positive rate may increase. When used before an injurious exposure has occurred, the test will not forecast AKI. Similarly, the test might not remain positive for a long time after injury [28]. If positive results are obtained, the test should be interpreted along with other clinical factors and nephrology consultation should be considered. When used properly, biomarker-guided interventions are useful in AKI prevention. This was demonstrated in a clinical trial including high risk patients, defined as urinary [TIMP-2]•[IGFBP7] > 0.3 undergoing cardiac surgery. In that study, implementation of the KDIGO guidelines, consisting of optimization of volume status and hemodynamics, avoidance of nephrotoxic drugs, and preventing hyperglycemia, resulted in an absolute risk reduction of 16.6% in the incidence of AKI compared with the standard care [29].

An important limitation of our study was the small sample size. Another study limitation was that patients with incomplete clinical files or those who were transferred to other hospitals due to local saturation were not included

in the study, and this may represent a selection bias. Considering that standardized definitions of AKI are based on sCr and urine output [30], then inaccessibility to nursing records restricted to COVID-19 areas represents an important study limitation because urine output was not used for diagnosis of AKI, and sCr was not adjusted for fluid-balance. The lack of pre-hospital baseline sCr measurements was also a study limitation because baseline sCr values were an estimation. One additional study limitation was that our study was conducted at a national referral center for respiratory diseases receiving disproportionately more patients with severe COVID-19, and this represents a potential source of referral bias.

Conclusions

Elevated values of urinary [TIMP-2]•[IGFBP7] were risk factors for AKI and persistent AKI was a risk factor for mortality. These biomarkers, together with clinical information, were useful for identification of subclinical AKI in critically ill COVID-19 patients. The role of additional biomarkers and their possible combinations for early detection of AKI in critically ill COVID-19 patients remains to be explored in large clinical trials. Preventable causes of AKI should be reduced.

Abbreviations

AKI, acute kidney injury; AUC, area under the receiver-operating characteristics curve; BMI, body mass Index; CKD, chronic kidney disease; COVID-19, Coronavirus disease 2019, eGFR, estimated glomerular filtration rate; HR, hazard ratio; ICU, chronic kidney disease; IGFBP7, insulin like growth factor binding protein 7; IMV, invasive mechanical ventilation; INER, National Institute of Respiratory Diseases; IQR, inter quartile range; KDIGO, Kidney Disease Improving Global Outcomes; NGAL, neutrophil gelatinase-associated lipocalin; PaO₂/FiO₂, partial arterial oxygen pressure/inspired oxygen fraction; pCO₂, partial pressure of carbon dioxide; PEEP, positive end-expiratory pressure; rRT-PCR, real-time reverse transcription–polymerase chain reaction; SARS- CoV-2, severe acute respiratory syndrome coronavirus 2; SOFA, sequential organ failure assessment; sCr, rerum creatinine; TIMP2, tissue inhibitor of metalloproteinases 2.

Declarations

Ethics approval and consent to participate: The Institutional Review Board approved the study (Approval No C26-20) and written informed consent was obtained from all participants.

Consent for publication: NA

Availability of data: All data generated and analyzed during this study are included in this published article and its supplementary information file [S1 File Raw Data].

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Validation: Rossana Olmedo-Ocampo; Visualization: Amy Peralta-Prado; Yara Luna Villalobos; Claudia Alvarado-de la Barrera; Writing-Original draft preparation: Claudia Alvarado-de la Barrera; Paola Fernández-Campos; Writing-Review and editing: Claudia Alvarado-de la Barrera.

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Figures

Figure 1

Study diagram. Numbers of individuals assessed for eligibility and individuals included in the study.

Figure 2

Cumulative AKI events according to urinary NGAL concentrations. AKI in individuals with urinary NGAL ≥ 45 ng/ml (blue line) vs. AKI in individuals with urinary NGAL <45 ng/ml (purple line) during hospitalization ($p=0.028$). Time 0 corresponded to hospital admission.

Figure 3

Cumulative AKI events according to urinary [TIMP-2]•[IGFBP7] concentrations. AKI in individuals with urinary [TIMP-2]•[IGFBP7] ≥ 0.2 (ng/ml)²/1000 (blue line) vs. AKI in individuals with urinary [TIMP-2]•[IGFBP7] < 0.2 (ng/ml)²/1000 (purple line) during hospitalization (p=0.013). Time 0 corresponded to hospital admission.

Figure 4

Survival in the AKI group and the non-AKI group. Survival in the AKI group (blue line) vs. survival in the non-AKI group (purple line) during hospitalization (p=0.019). Time 0 corresponded to hospital admission.