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# **Acute kidney injury in critical COVID-19 patients: usefulness of urinary biomarkers and kidney proximal tubulopathy**

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## **KEYWORDS:**

NGAL; TIMP-2; IGFBP7; NephroCheck; kidney proximal tubulopathy; Fanconi syndrome; proteinuria; AKI; covid-19; SARS-CoV-2

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# ABSTRACT

Tubular injury is the main cause of acute kidney injury (AKI) in critically ill COVID-19 patients. Proximal tubular dysfunction (PTD) and changes in urinary biomarkers, such as NGAL, TIMP-2, and IGFBP7 product ([TIMP-2]•[IGFBP7]), could precede AKI. We conducted a prospective cohort study from 2020/03/09 to 2020/05/03, which consecutively included all COVID-19 patients who had at least one urinalysis, to assess the incidence of PTD and AKI, and the effectiveness of PTD, NGAL, and [TIMP-2]•[IGFBP7] in AKI and persistent AKI prediction using the area under the receiver operating characteristic curves (AUCs), Kaplan–Meier methodology (log-rank tests), and Cox models. Among the 60 patients admitted to the ICU with proven COVID-19 (median age: 63-year-old (interquartile range: IQR, 55–74), 45 males (75%), median simplified acute physiology score (SAPS) II: 34 (IQR, 22–47) and median BMI: 25.7 kg/m<sup>2</sup> (IQR, 23.3–30.8)) analyzed, PTD was diagnosed in 29 patients (48%), AKI in 33 (55%) and persistent AKI in 20 (33%). Urinary NGAL had the highest AUC for AKI prediction: 0.635 (95%CI: 0.491–0.779) and persistent AKI prediction: 0.681 (95%CI: 0.535–0.826), as compared to PTD and [TIMP-2]•[IGFBP7] (AUCs <0.6). AKI was independently associated with higher SAPSII (HR = 1.04, 95%CI: 1.01–1.06, p = 0.005) and BMI (HR = 1.07, 95%CI: 1.00–1.14, p = 0.04) and persistent AKI with higher SAPSII (HR = 1.03, 95%CI: 1.00–1.06, p = 0.048) and nephrotoxic drug use (HR = 3.88, 95%CI: 1.20–12.5, p = 0.02). In conclusion, in critically ill COVID-19 patients, the incidence of PTD and AKI was relatively high. NGAL was the best urinary biomarker for predicting AKI, but only clinical severity was independently associated with its occurrence.

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## Introduction

Two years after the pandemic, coronavirus disease 2019 (COVID-19) has been reported to be responsible for approximately 18 million deaths worldwide [1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) leads to asymptomatic or paucisymptomatic infections as well as severe or critical COVID-19. Among patients admitted to the intensive care unit (ICU) for COVID-19, acute kidney injury (AKI) is a prevalent complication (in approximately one-third of patients), associated with worse outcomes [2–4].

However, the pathophysiology of COVID-19-associated AKI remains incompletely elucidated [3]. Several hypotheses have been proposed, including kidney tropism and direct damage by the virus, SARS-CoV-2 induced cytokine storm and/or coagulopathy, organ crosstalk, shock, nephrotoxic drugs, and high levels of positive end-expiratory pressure

(PEEP) [3,4]. Although specific kidney diseases have been reported, such as collapsing glomerulopathy, acute tubular necrosis appears to be the predominant renal pathology [4]. Several studies have highlighted kidney proximal tubular dysfunction in COVID-19 patients [5–7] and have suggested that it could precede AKI [7]. Thus, tubular dysfunction could play a critical role by facilitating an early prediction of COVID-19-associated AKI, as a urinary biomarker of kidney damage or stress [8]. Kidney damage biomarkers, such as urinary neutrophil gelatinase-associated lipocalin (NGAL) [9–12], and kidney stress biomarkers, such as tissue inhibitor of metalloproteinases-2 and insulin-like growth factor binding protein 7 product ([TIMP-2]•[IGFBP7]) [12,13], have been reported, in a few studies, to be good predictors of AKI in COVID-19 patients. However, none of these studies has compared their diagnostic effectiveness.

This study aimed to assess the incidence of proximal tubular dysfunction and AKI in COVID-19 patients and the effectiveness of urinary biomarkers, including tubular parameters, NGAL, and [TIMP-2]•[IGFBP7], in predicting COVID-19-associated AKI in ICU patients.

## **Patients and methods**

### **Study design and settings**

This single-center observational cohort study was conducted at the Intensive Care Medicine Department of Montpellier University Hospital. Investigators prospectively collected clinical and biological data for all consecutive critically ill COVID-19 patients admitted to the ICU from 9 March 2020 to 3 May 2020.

Patients were followed up until hospital discharge, then data were analyzed retrospectively.

### **Patients**

All consecutive adult patients who were hospitalized during the study period were screened for ICU admission. SARS-CoV-2 pneumonia was confirmed by RT-PCR on nasopharyngeal swabs or bronchoalveolar lavage, combined with suggestive abnormalities on chest radiography or CT-scan. Patients aged <18-year-old or pregnant and those with suspected COVID-19 not confirmed by SARS-CoV-2 RT-PCR were excluded. Patients on maintenance dialysis, those with stage 4–5 chronic kidney disease [14] at admission, and those who did not undergo urinalysis at admission were also excluded.

### **Data collection**

Demographic and clinical data, including comorbidities, were collected, and the Charlson Comorbidity Index was calculated [15]. Disease severity was assessed using the Simplified Acute Physiology Score (SAPS) II, and the worst oxygen arterial partial pressure to the fraction of inspired oxygen ratio (PaO<sub>2</sub>/FiO<sub>2</sub>) 24 h after ICU admission was calculated [16,17].

Blood and urine samples were collected upon ICU admission. Samples were immediately processed, except for urinary NGAL, TIMP-2, IGFBP7, and amino acids, which were further processed on urinary samples frozen at  $-20^{\circ}\text{C}$ .

Almost all chemical lab measurements, including the NGAL test, were performed on a Cobas 8000 instrument using the e802, c702, and c502 modules (Roche Diagnostics, Meylan, France). Plasma creatinine was measured by an isotope dilution mass spectrometry (IDMS) traceable enzymatic method on Cobas 8000 instrument.

Urine TIMP-2 and IGFBP7 levels were measured using a Vitros® 3600 Immunodiagnostic System (Ortho Clinical Diagnostics, Rochester, NY, USA) on urine samples of at least 100  $\mu\text{L}$ . The TIMP-2 and IGFBP7 product ( $[\text{TIMP-2}] \cdot [\text{IGFBP7}]$ ), also known as NephroCheck® score, was calculated according to the formula:  $([\text{TIMP-2}] \times [\text{IGFBP7}])/1000$ .

Amino acids in the urine samples were quantified by ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) on an Acquity UPLC system coupled to a XevoTQD (Waters Corporation, Milford, MA, USA). Ion-pairing chromatography was performed on a BEH C18 1.7  $\mu\text{m}$  column (Waters®) at a flow rate of 0.65 mL/min using mobile phases containing tridecafluoroheptanoic acid (Sigma-Aldrich, St. Louis, Missouri, USA) and Sigma® calibrators. The amino acid concentration in the urine was normalized to urine creatinine (in mmol/L) and compared to the values of normal subjects ( $N = 80$ , without tubulopathy, or COVID-19).

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula was used to estimate glomerular filtration rate (GFR) [18]. The renal phosphate threshold/GFR (TmP/GFR) ratio was calculated as follows [19]: (i) calculation of the renal tubular reabsorption of phosphate (RTP) using the following formula:  $\text{RTP} = 1 - [\text{phosphate clearance}/\text{creatinine clearance}] \times 100$ , and (ii) if  $\text{RTP} \leq 0.86$ :  $\text{TmPi}/\text{GFR} = \text{RTP} \times \text{serum phosphate (mmol/L)}$  and  $\text{RTP} > 0.86$ :  $\text{TmPi}/\text{GFR} = 0.3 \times \text{RTP}/[1 - (0.8 \times \text{RTP})] \times \text{serum phosphate (mmol/L)}$ .

## Definitions

Proximal tubular dysfunction was defined as the association of at least two of the following six criteria:

- urinary protein/creatinine ratio  $> 0.2$  g/g with urinary albumin to protein ratio  $< 0.5$ ;
- amino acids in the urines;
- urinary levels of  $\beta 2$ -microglobulin  $> 0.3$  mg/L [20];
- hypouricemia (serum uric acid level  $< 220$   $\mu\text{mol/L}$  in male and  $< 184$   $\mu\text{mol/L}$  in female) with inappropriate uricosuria (fractional excretion of uric acid  $> 10\%$ )
- hypophosphatemia (serum phosphate level  $< 0.81$  mmol/L) with inappropriate phosphaturia (fractional excretion of phosphate  $> 20\%$ ) or renal phosphate leak ( $\text{TmP}/\text{GFR} < 0.77$  mmol/L [21]);

• normoglycemic glycosuria (glycosuria  $>0.15$  g/L and glycemia  $<1.80$  g/L). AKI was defined according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guide-lines [22].

All patients were classified on day seven after ICU admission.

Baseline serum creatinine level was defined as the usual value of serum creatinine level preceding admission within at least 7 days.

Persistent AKI was defined as a steady or higher AKI KDIGO stage and/or persistence of oliguria after day 2 of AKI onset [23].

## **Outcomes**

Hospital and ICU lengths of stay were recorded. Vital status at ICU and hospital discharge were collected, and mortality rates were calculated.

## **Statistical analysis**

Categorical data were described as numbers and percentages and continuous data as medians with the 25th and 75th per-centiles (interquartile range: IQR). Patients were divided into two groups: those with and without AKI. Categorical variables were compared using the Chi-square or Fisher's exact test, and continuous variables were compared using Student's t-test or Wilcoxon's rank-sum test, as appropriate.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for AKI prediction were calculated, and receiver operating characteristic (ROC) curves were plotted for proximal tubular dysfunction, [TIMP-2]•[IGFBP7], and urinary NAGL. The area under the ROC curve (AUC) for AKI prediction was calculated. Optimal cutoff values were estimated by maximizing the Youden index. The same calculations were performed for biomarkers of persistent AKI.

The population was then divided into groups according to the cutoff value for each biomarker. Cumulative incidence curves of AKI and persistent AKI were obtained using the Kaplan–Meier method and compared using the log-rank test.

Factors associated with AKI (and persistent AKI) were assessed using univariate and multivariate Cox regression models. A conditional stepwise regression with 0.1 a critical p-value in the univariate analysis for entry into the multivariable analysis was performed to select the most informative variables. In the case of interactions and correlations between the explanatory variables, only the most clinically relevant variables were included in the final model (for example, we found an interaction between norepinephrine requirements, invasive mechanical ventilation requirements, and SAPSII. Therefore, we included SAPS II in the model because it reflects both the severity of respiratory and hemodynamic failure). In the case of skewed distributions, biomarker values were log-transformed before modeling. The proportional hazard assumption was assessed by inspecting scaled Schoenfeld residuals. The results of the Cox regression models are reported as HR or aHR with 95%CI.

All tests were two-sided, and a p-value less than 0.05 was considered statistically significant. The R software version 4.2.0 (The R Foundation for Statistical Computing, Vienna, Austria) was used for the analyses.

## Ethics

The Institutional Review Board of Montpellier University Hospital approved the study protocol (IRB-MTP\_2020\_05\_202000482, date of approval: 20/05/2020) and waived the need for signed patient consent. The study protocol was registered at clinicaltrials.gov (NCT04393428), and the study was conducted in accordance with the Declaration of Helsinki.

## Results

### Patients

Among 170 patients admitted to the ICU during the study period, 77 had a proven COVID-19, and 60 (45 males, 75%) were included in the study (Figure 1). The characteristics of the study population at ICU admission and during COVID-19 management are shown in Table 1. The median age and BMI were 63-year-old (IQR: 55–74) and 25.7 kg/m<sup>2</sup> (IQR: 23.3, 30.8), respectively, and the median Charlson index was 2 (IQR: 1–4). Importantly, 9 (15%) patients were followed up for chronic kidney disease, and 23 (38%) received a nephrotoxic drug before or immediately after ICU admission.

During the first 24 h of ICU stay, the median SAPS II and worst PaO<sub>2</sub>/FiO<sub>2</sub> were 34 (IQR: 22, 47) and 120 mmHg (IQR: 80, 172), respectively. All patients received oxygen support, and almost half (29 patients, 48%) were intubated and mechanically ventilated. More than half of the patients (n = 33, 55%) required vasopressors. All patients who required vasopressors were mechanically ventilated and received a low dose (<1 µg/kg/min) of norepinephrine, except for two with septic shock. The median daily urine output was 800 mL (IQR: 600–1100 mL).

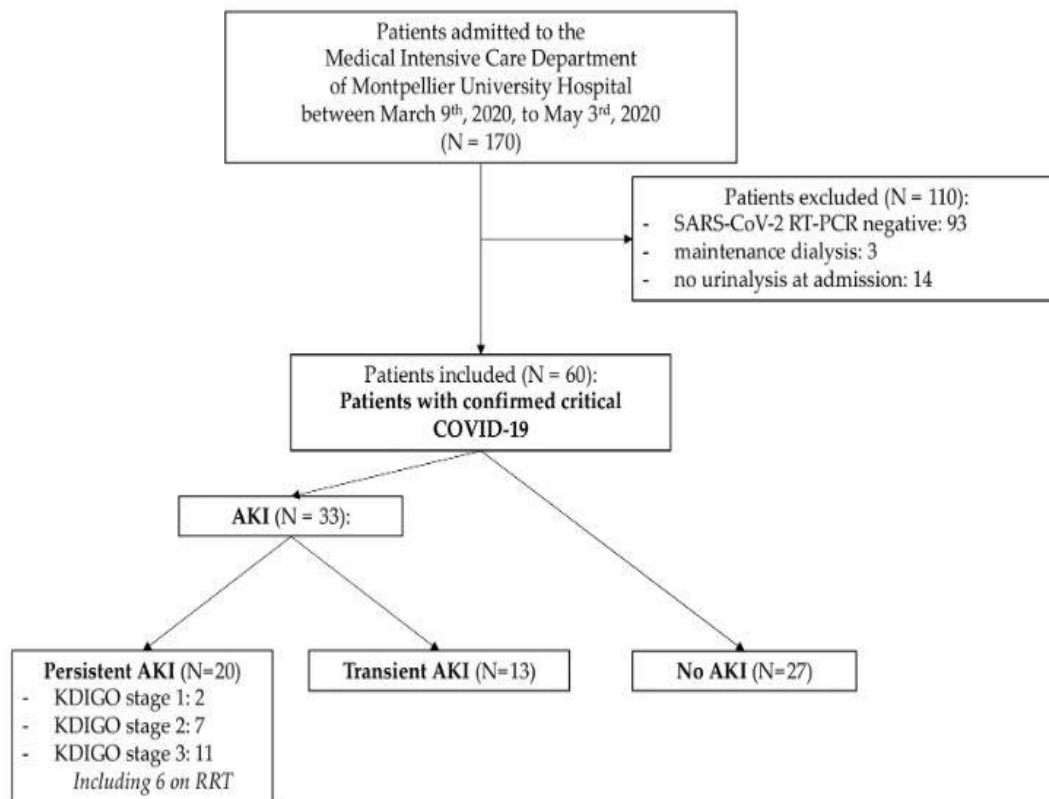
At ICU admission, the median C-reactive protein (CRP) level and lymphocyte count were 136 mg/L (IQR: 67, 215) and 870 cells/mm<sup>3</sup> (IQR: 570, 885), respectively. The median serum creatinine levels at admission and at baseline were 81 µmol/L (IQR: 59, 103) and 74 µmol/L (IQR: 57, 79), respectively.

Proximal tubular dysfunction was diagnosed in 29 COVID-19 patients (48%) upon ICU admission. Most patients had partial Fanconi syndrome, as only 3 (5%) presented with 6/6 features of proximal tubular dysfunction on urinalyses and 8 (13%) with 5. The median values of urinary NGAL and [TIMP-2]•[IGFBP7] were 56 ng/mL (IQR:50–88) and 0.73 (IQR:0.32, 1.59), respectively (Table 1).

Features of ICU admission urinalyses are summarized in Figure 2.

During the ICU stay, more than half of the patients (33 patients, 55%) developed AKI according to the 2012 KDIGO classification. Among them, 20 patients (33%) developed persistent AKI, KDIGO stage 1 in 2 patients (10%), stage 2 in 7 (35%), and stage 3 in 11 (55%); renal replacement therapy (RRT) was initiated in 6 patients (18%) (Figure 1). Interestingly, of the 27 patients without AKI, 11 had elevated NGAL, 11 had elevated [TIMP-2]-[IGFBP7] and 13 had PTD.

The risks for AKI and persistent AKI were estimated at 55.7% (confidence interval at 95%:95%CI: 41.0 – 66.6) and 33.3% (95%CI: 20.3 – 44.3), respectively (Figure 3).



**Figure 1.** Flow chart of the study population. AKI: acute kidney injury, KDIGO: kidney disease: improving global outcomes; RRT: renal replacement therapy.

## Outcomes

The median lengths of stay in the ICU and hospital were 9 days (IQR: 6, 15) and 12 days (IQR: 8, 23), respectively. Thirty-three patients (22%) died during the hospital stay, and among those discharged alive, 5 patients (8%) still required RRT.

## Urinalysis for AKI and persistent AKI prediction

At ICU admission, the AUCs for AKI prediction of NGAL, [TIMP-2]•[IGFBP7], and proximal tubular dysfunction were 0.619 (95%CI: 0.479 – 0.759), 0.593 (95% CI: 0.445 – 0.740), and 0.470 (95%CI: 0.325 – 0.614), respectively. The AUCs for persistent AKI prediction for these three biomarkers were 0.681 (95%CI: 0.535 – 0.826), 0.569 (95%CI: 0.405 – 0.733), and 0.547 (95%CI: 0.395 – 0.699), respectively.



ROC curves are displayed in Figure 4, and Table 2 summarizes the effectiveness of the biomarkers of kidney stress, kidney damage, and proximal tubular dysfunction in predicting AKI and persistent AKI.

Urinary NGAL had the highest sensitivity (66.7%) and NPV (59.3%) for AKI prediction. [TIMP-2]•[IGFBP7] had the highest PPV (67.7%), whereas the presence of four or more signs of proximal tubular dysfunction had the highest specificity (96.3%) for AKI prediction (Appendix A1).

In addition, proximal tubular dysfunction showed the highest sensitivity (85.0%) for persistent AKI prediction but the lowest specificity (33.5%). In contrast, NGAL showed the highest specificity (67.5%) and sensitivity (65%). The NPVs ranged from 76.7 to 85.7%, while all factors had low PPVs ( $\leq 50\%$ ) for predicting persistent AKI.

Interestingly, only urinary NGAL was able to discriminate between patients with AKI and persistent AKI on day 7 ( $p = 0.02$  and  $p = 0.03$ , respectively) (Figure 5). On the contrary, the incidence of AKI events was not significantly different in patients with [TIMP-2]•[IGFBP7]  $\geq 0.7$  ( $p = 0.08$  and  $p = 0.2$ ), in those who presented with proximal tubular dysfunction ( $p = 0.7$  and  $p = 0.6$ ), or with a urine protein/creatinine ratio (UPCR)  $\geq 50$  mg/g ( $p = 0.4$  and  $p = 0.6$ ) (Appendices A2–A5).

### **Factors associated with AKI and persistent AKI**

In the univariate analysis patients with AKI had higher value of BMI (hazard ratio: HR = 1.06, 95%CI: 1 – 1.13,  $p = 0.034$ ), baseline creatinine (HR = 1.02, 95%CI: 1.01 – 1.03,  $p = 0.003$ ), Charlson index (HR = 1.15, 95%CI: 1.03 – 1.29,  $p = 0.011$ ), and SAPS II (HR = 1.04, 95%CI: 1.03 – 1.06,  $p < 0.001$ ), and lower PaO<sub>2</sub>/FiO<sub>2</sub> ratio (HR = 0.99, 95%CI: 0.982 – 0.996,  $p = 0.002$ ) and lymphocyte count (HR = 0.06, 95%CI: 0.01 – 0.31,  $p < 0.001$ ), and history of chronic kidney disease (HR = 3.99, 95%CI: 1.77 – 8.99,  $p < 0.001$ ). Moreover, AKI was associated with the need for invasive mechanical ventilation (HR = 4.09, 95%CI: 1.88 – 8.88,  $p < 0.001$ ) or norepinephrine (HR = 2.79, 95%CI: 1.76 – 4.42,  $p < 0.001$ ). Importantly, NGAL levels  $\geq 53$  ng/mL were associated with AKI (HR = 2.27, 95%CI: 1.10 – 4.69,  $p = 0.027$ ), whereas [TIMP-2]•[IGFBP7] (HR = 1.89, 95%CI: 0.927 – 3.85,  $p = 0.08$ ) were not.

The variables associated with persistent AKI in the univariate analysis were similar to those associated with AKI. However, we found that AKI was independently associated only with higher SAPS II (adjusted HR: aHR = 1.04, 95%CI: 1.01–1.06,  $p = 0.005$ ) and higher BMI (aHR = 1.07, 95%CI: 1.00–1.14,  $p = 0.04$ ) by multivariable analysis.

In the multivariable analysis, only higher SAPS II (aHR = 1.03, 95%CI: 1.00–1.06,  $p = 0.048$ ) and nephrotoxic treatment before ICU admission (aHR = 3.88, 95%CI: 1.20–12.5,  $p = 0.02$ ) were associated with persistent AKI.

**Table 1.** Characteristics of the study population and COVID-19 management.

Characteristic	All (N=60) <sup>a</sup>	AKI (N=33) <sup>a</sup>	No AKI (N=27) <sup>a</sup>	p-Value <sup>b</sup>
Age (years)	63 (55, 74)	66 (55, 75)	62 (54, 68)	0.2
Sex (male)	45 (75%)	25 (76%)	20 (74%)	0.9
Body mass index (kg/m <sup>2</sup> )	25.7 (23.3, 30.8)	27.8 (24.2, 31.1)	24.5 (21.3, 29.2)	0.054
<b>Medical history</b>				
Hypertension	26 (43%)	18 (55%)	8 (30%)	0.053
Cardiovascular disease	20 (33%)	13 (39%)	7 (26%)	0.3
Diabetes	19 (32%)	12 (36%)	7 (26%)	0.4
Chronic kidney disease	9 (15%)	8 (24%)	1 (4%)	0.033*
Baseline creatinine, µmol/L	74 (57, 90)	81 (57, 97)	65 (55, 87)	0.14
Charlson index	2 (1, 4)	3 (1, 5)	2 (1, 2)	0.022*
Nephrotoxic medication	19 (32%)	11 (33%)	8 (30%)	0.8
Angiotensin converting enzyme inhibitors	14 (23%)	7 (21%)	7 (26%)	0.7
Angiotensin II receptor blocker	5 (8%)	4 (12%)	1 (4%)	0.4
<b>COVID-19 features at ICU admission</b>				
Lymphocytes, G/L	0.87 (0.57, 1.09)	0.73 (0.52, 0.97)	0.99 (0.82, 1.16)	0.007*
D-dimer, U/L	864 (540, 1,530)	890 (572, 1,751)	779 (526, 1,174)	0.6
C-reactive protein, mg/L	136 (67, 215)	152 (105, 214)	97 (45, 209)	0.1
Lactate, mmol/L	1.35 (1.08, 1.80)	1.20 (0.98, 1.73)	1.55 (1.30, 2.03)	0.13
Creatinine, µmol/L	80 (59, 124)	96 (65, 172)	74 (58, 90)	0.040*
Urine output in the first 24h, ml	800 (600, 1,100)	800 (700, 1,100)	900 (600, 1,050)	0.9
UPCR, mg/g	50 (27, 92)	61 (36, 108)	45 (22, 73)	0.074
UPCR ≥50 mg/g	30 (50%)	19 (58%)	11 (41%)	0.2
Proximal tubular dysfunction	29 (48%)	16 (48%)	13 (48%)	>0.9
Urinary NGAL, ng/ml	56 (50, 88)	67 (50, 117)	50 (50, 66)	0.1
Urinary NGAL ≥53 ng/ml	33 (55%)	22 (67%)	11 (41%)	0.045*
[TIMP-2]•[IGFBP7]	0.74 (0.32, 1.59)	0.91 (0.35, 1.78)	0.51 (0.27, 1.21)	0.2
[TIMP-2]•[IGFBP7] ≥0.7	32 (53%)	21 (64%)	11 (41%)	0.077
<b>Disease severity within 24h ICU</b>				
SAPS II	34 (22, 46)	42 (34, 49)	21 (17, 30)	<0.001*
PaO <sub>2</sub> /FIO <sub>2</sub>	120 (80, 172)	90 (75, 119)	167 (135, 211)	<0.001*
<b>COVID-19 management</b>				
Invasive mechanical ventilation	29 (48%)	24 (73%)	5 (19%)	<0.001*
Prone positioning	11 (18%)	10 (30%)	1 (4%)	0.016*
ECMO	2 (3.3%)	2 (6.1%)	0 (0%)	0.5
Norepinephrine	26 (43%)	23 (70%)	3 (11%)	<0.001*
Renal replacement therapy	6 (10%)	6 (18%)	0 (0%)	0.028*
Nephrotoxic during ICU stay	10 (17%)	5 (15%)	5 (19%)	0.4
<b>Change in serum creatinine</b>				
From baseline to maximum, µmol/L	8 (0, 12)	43 (27, 124)	11 (-20, 25)	<0.001*
From baseline to discharge, µmol/L	-1 (-16, 4)	14 (5, 46)	1 (-18, 21)	0.062
ICU length of stay	9 (6, 14)	10 (6, 18)	7 (4, 10)	0.069
Hospital length of stay	12 (8, 23)	17 (10, 26)	10 (8, 14)	0.035*
Mortality	13 (22%)	9 (27%)	4 (15%)	0.2

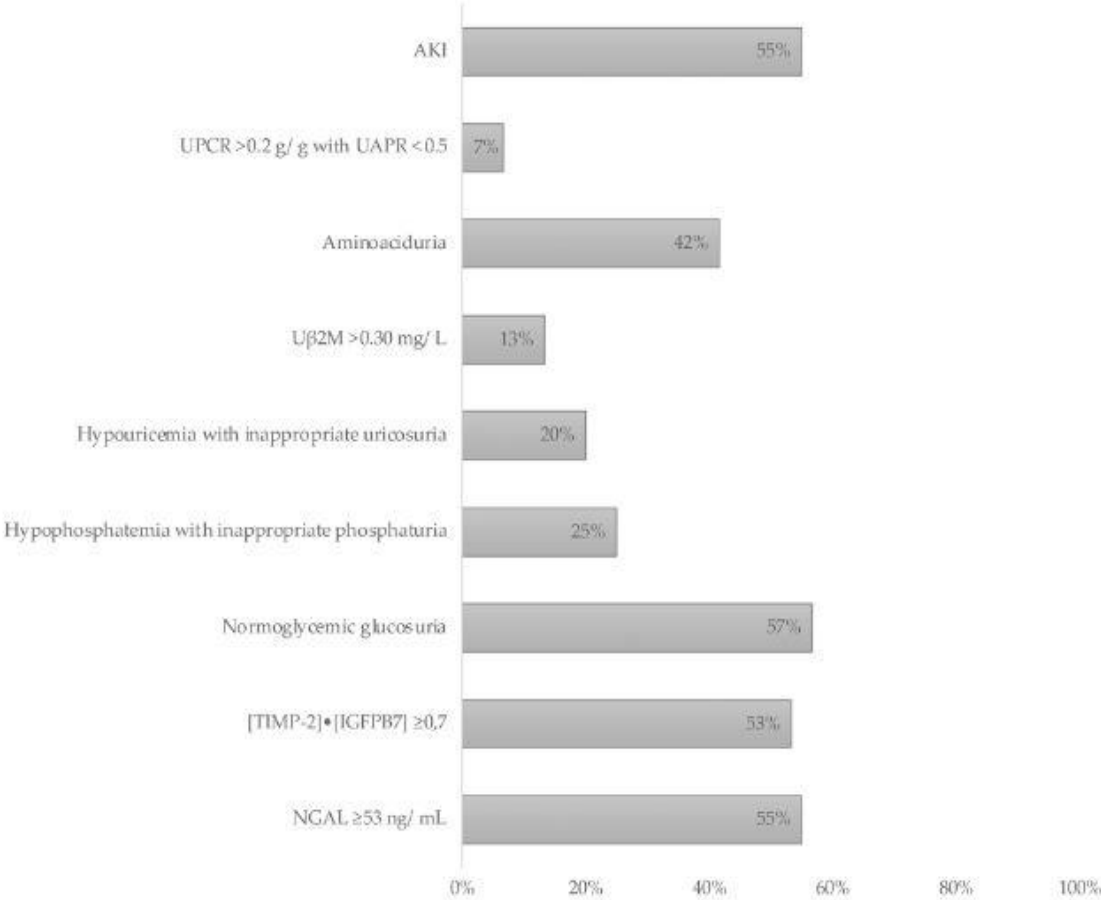
<sup>a</sup>Median and interquartile range (IQR); number of patients (N) and percentage (%); <sup>b</sup>Wilcoxon rank sum exact test; Pearson's chi-squared test; Wilcoxon rank sum test; Fisher's exact test; \*Significant p-Value. [TIMP-2]•[IGFBP7] was calculated as [TIMP-2]×[IGFBP7]/1000. ECMO: extracorporeal membrane oxygenator; ICU: intensive care unit; IGFBP7: insulin-like growth factor binding protein 7; NGAL: neutrophil gelatinase-associated lipocalin; PaO<sub>2</sub>/FIO<sub>2</sub>: oxygen arterial partial pressure to the fraction of inspired oxygen ratio; PEEP: positive end-expiratory pressure; SAPS II: simplified acute physiology score II; TIMP-2: tissue inhibitor of metalloproteinases 2; UPCR: urine protein/creatinine ratio.

The results of univariate and multivariable analyses are presented in Table 3.

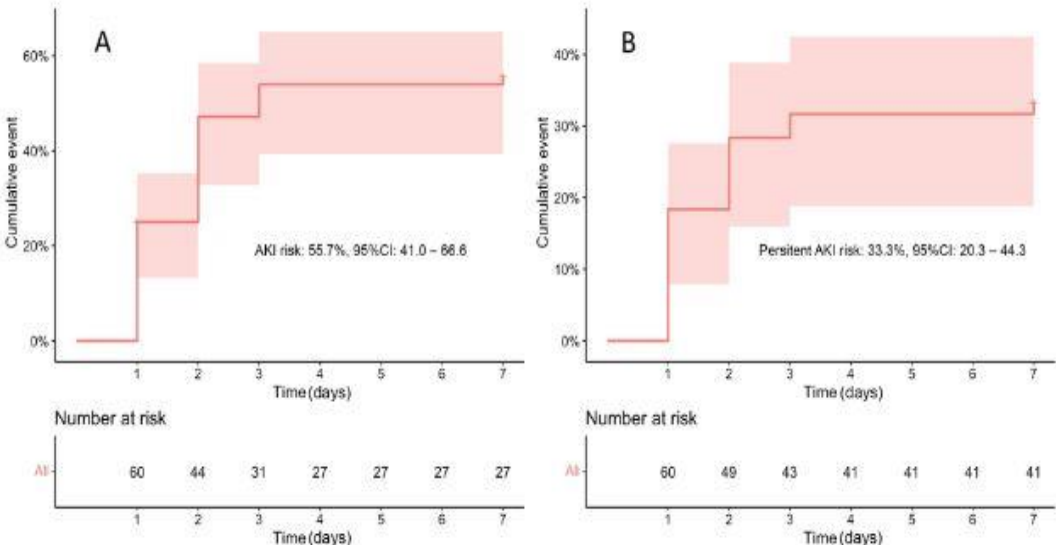
## Discussion

Here, we report the results of a cohort study including 60 critically ill patients with COVID-19, showing that 48% presented with proximal tubular dysfunction, 55% had AKI, and 33% had persistent AKI. In patients with COVID-19, urinary NGAL on ICU admission had a better effectiveness in predicting AKI and persistent AKI on day 7 than [TIMP-2]•[IGFBP7] and proximal tubular dysfunction. However, multivariable analyses showed that only increased SAPS II and BMI and use of angiotensin-converting enzyme inhibitors (ACEI) or

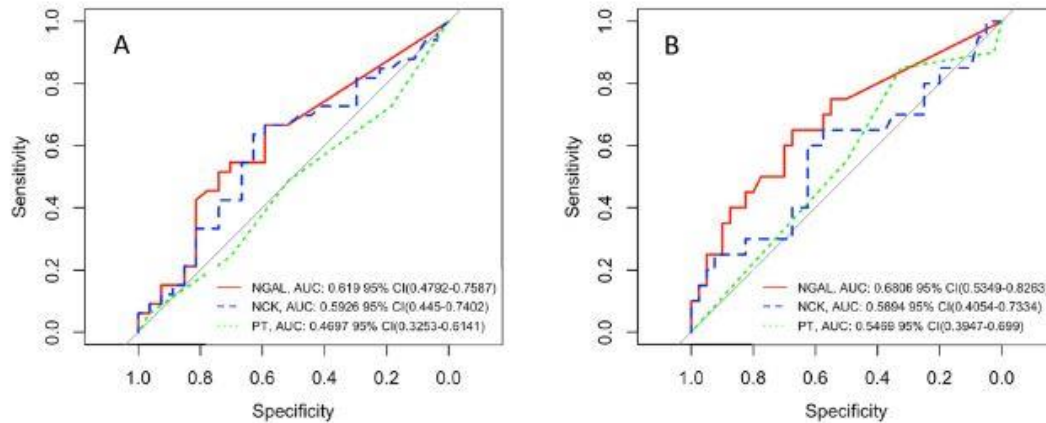
angiotensin II receptor blockers (ARB) were independently associated with AKI or persistent AKI.



**Figure 2.** Prevalence (%) of acute kidney injury (AKI), proximal tubular dysfunction features, and elevated level of kidney stress biomarkers ([TIMP-2]•[IGFBP7]) and kidney damage biomarker (NGAL) in urine of COVID-19 patients at ICU admission. IGFBP7: insulin like growth factor binding protein 7; NGAL: neutrophil gelatinase-associated lipocalin; TIMP2: tissue inhibitor of metalloproteinases 2.



**Figure 3.** Cumulative incidence of: (A) acute kidney injury (AKI) and (B) persistent AKI in COVID-19 patients and confidence Interval at 95% (95%CI).

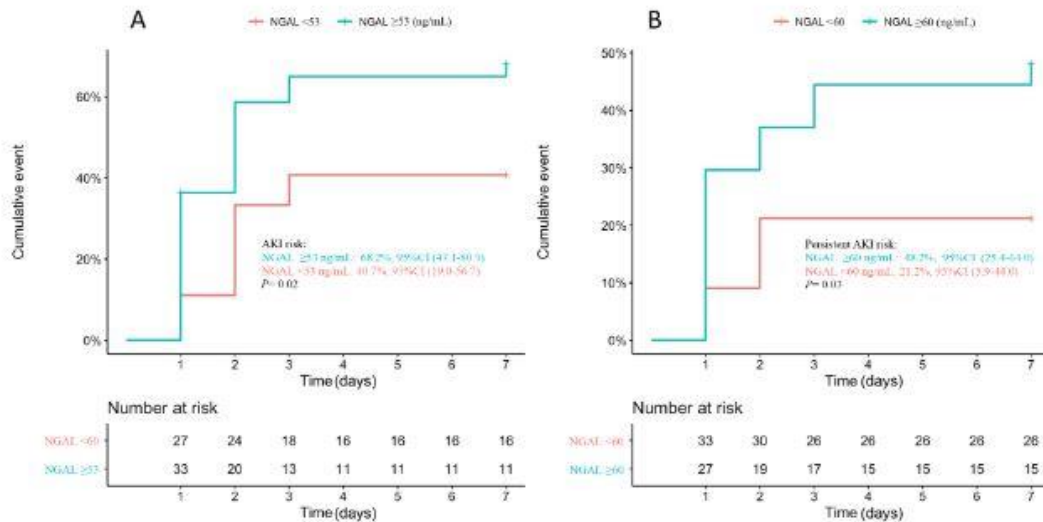


**Figure 4.** Receiver operating characteristic (ROC) curves of urinary neutrophil gelatinase-associated lipocalin (NGAL, red line), [TIMP-2]-[IGFBP7] (NCK, blue dashed line), and occurrence of kidney proximal tubulopathy (PT, green dotted line) at ICU admission for predicting: (A) acute kidney injury (AKI) and (B) persistent AKI in COVID-19 patients. AUC: area under the ROC curve, 95%CI: confidence interval at 95%.

**Table 2.** Diagnostic effectiveness of urinary biomarkers for acute kidney injury (AKI) and persistent AKI in critical COVID-19 patients.

Characteristic	Se (%)	Sp (%)	PPV (%)	NPV (%)	AUC	95% CI
<b>AKI</b>						
NGAL ( $\geq 53$ ng/mL)	66.7	59.3	66.7	59.3	0.619	0.479–0.759
[TIMP-2]-[IGFBP7] ( $\geq 0.7$ )	63.6	63.0	67.7	58.6	0.593	0.445–0.740
Proximal tubular dysfunction	48.5	51.6	55.2	45.2	0.470	0.325–0.614
<b>Persistent AKI</b>						
NGAL ( $\geq 60$ ng/mL)	65.0	67.5	50.0	79.4	0.681	0.535–0.826
[TIMP-2]-[IGFBP7] ( $\geq 0.7$ )	65.0	57.5	43.3	76.7	0.569	0.405–0.733
Proximal tubular dysfunction	85.0	32.5	38.6	81.3	0.547	0.395–0.699

95% CI: confidence interval at 95%; AUC: area under the receiver operating curve; IGFBP7: insulin-like growth factor binding protein 7; NPV: negative predictive value; PPV: positive predictive value; Se: sensitivity; Sp: specificity; TIMP-2: tissue inhibitor of metalloproteinases 2; NGAL: neutrophil gelatinase-associated lipocalin; [TIMP-2]-[IGFBP7] was calculated as follows:  $[\text{TIMP-2}] \times [\text{IGFBP7}] / 1000$ .



**Figure 5.** Cumulative incidence of: (A) acute kidney injury (AKI) and (B) persistent AKI in COVID-19 patients according to cutoff values of urinary neutrophil gelatinase-associated lipocalin (NGAL).

**Table 3.** Factors associated with acute kidney injury (AKI) and persistent AKI.

Characteristic	Univariate analysis			Multivariable analysis		
	HR	95%CI	p-Value	aHR	95%CI	p-Value
<b>AKI</b>						
BMI (/kg/m <sup>2</sup> )	1.06	1.00-1.13	0.034*	1.07	1.00-1.14	0.041*
Charlson Index (/point)	1.15	1.03-1.29	0.01*	1.01	0.87-1.16	0.95
SAPS II (/point)	1.04	1.03-1.06	<0.001*	1.04	1.02-1.06	0.001*
Lymphocyte count (/log 100 cells/mm <sup>3</sup> )	0.06	0.01-0.31	<0.001*	0.47	0.09-2.52	0.38
NGAL $\geq 53$ ng/mL	2.27	1.10-4.69	0.027*	1.24	0.54-2.85	0.61
[TIMP-2]-[IGFBP7] $\geq 0.7$	1.22	0.55-2.68	0.08	1.26	0.57-2.78	0.57
<b>Persistent AKI</b>						
Nephrotoxic medication	2.54	1.06-6.11	0.04*	3.88	1.20-12.5	0.02*
Charlson Index (/point)	1.28	1.13-1.46	<0.001*	1.09	0.90-1.32	0.40
SAPS II (/point)	1.04	1.02-1.06	<0.001*	1.04	1.00-1.07	0.048*
Lymphocyte count (/log 100 cells/mm <sup>3</sup> )	0.06	0.01-0.28	0.002*	0.33	0.03-3.26	0.35
NGAL $\geq 60$ ng/mL	2.71	1.08-6.79	0.03*	1.15	0.40-3.25	0.80
[TIMP-2]-[IGFBP7] $\geq 0.7$	1.73	0.69-4.33	0.24			

\*Statistically significant. 95% CI: confidence interval at 95%; aHR: adjusted hazard ratio; BMI: body mass index; HR: hazard ratio; IGFBP7: insulin-like growth factor binding protein 7; NGAL: neutrophil gelatinase-associated lipocalin; SAPS: simplified acute physiology score; TIMP-2: tissue inhibitor of metalloproteinases 2. Nephrotoxic medication included angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) before ICU admission. [TIMP-2]-[IGFBP7] was calculated as [TIMP-2]  $\times$  [IGFBP7]/1000.

At the beginning of the pandemic, proteinuria, hematuria, and leukocyturia have been investigated as biomarkers for the early recognition of COVID-19-associated AKI [8, p.19], and proteinuria has been reported to be the most interesting [24–26]. However, its effectiveness in diagnosing COVID-19-associated AKI remains controversial because most patients infected with SARS-CoV-2 have proteinuria [24,27]. A specific kidney proximal tubular dysfunction (Fanconi syndrome) associated with SARS-CoV-2 infection has been reported to be the primum movens of COVID-19 associated AKI [7] and is associated with poor prognosis [6]. In accordance with previous studies [5–7], approximately half of our patients had Fanconi syndrome; however, its occurrence poorly predicted AKI and persistent AKI. Like HIV, SARS-CoV-2 attacks the kidney in different ways [28]. Different patterns have been reported: (i) nephrotic proteinuria suggestive of COVID-19-associated nephropathy (COVAN), (ii) acute glomerular injury with non-nephrotic proteinuria, and (iii) acute tubular

injury associated with tubular proteinuria related to proximal tubulopathy [4,24]. Alterations in renal function may be associated with any of these clinical profiles.

The use of kidney stress and damage biomarkers as predictors of COVID-19-associated AKI has been suggested previously [9–13]. This increase may reflect acute tubule injury that precedes loss of renal function [29]. Two studies [12,13] assessing [TIMP-2]•[IGFBP7] for the early recognition of COVID-19-associated AKI reported conflicting results. Husain-Syed et al. [13] found that higher levels of [TIMP-2]•[IGFBP7] were associated with worse outcomes (need for RRT and death). Casas-Aparicio et al. [12] have reported the effectiveness of [TIMP-2]•[IGFBP7] in predicting COVID-19-associated AKI. However, our results demonstrated the effectiveness of NGAL in predicting early COVID-19-associated AKI [9–12] and that NGAL outperformed [TIMP-2]•[IGFBP7] [12]. NGAL has also been reported as a prognostic factor for RRT requirement and death in COVID-19 patients [11,12,30]. Of note, our NGAL threshold of 53 ng/mL is in line with those reported previously (45 ng/mL) using an enzyme-linked immunosorbent assay (ELISA) [9,12], whereas thresholds of 118.8 or 150 ng/mL have been reported using NGAL dipstick [9,10,30].

In our patients, the occurrence of AKI was independently associated only with BMI [31], ACEI or ARB use [32,33], and most importantly with COVID-19 severity [34,35] whereas elevation of tubular injury biomarkers was associated with AKI only in univariate analysis. Indeed, NGAL elevation is most likely related to the activation of the immune system [36] explaining that urinary biomarkers, including NGAL, are not independently associated with AKI [9–12,30]. In the same vein, there is an association between elevated NGAL and mortality in COVID-19 patients, confirming its link with inflammation and severity [9,10] and also the role of inflammation in the development of AKI [37,38].

Our observations corroborate the existence of a continuum in the spectrum of COVID-19 associated AKI [4,24,39]. Indeed, some patients may present only subclinical AKI syndrome characterized by significant tubule injury (particularly, changes in the protein endocytosis machinery in proximal tubule epithelial cells) without changes in the glomerular filtration rate [12,37,40,41] while others may exhibit AKI related to various mechanisms [4,24,39]. This approach may explain why we found that proximal tubular dysfunction was

a sensitive marker for detecting persistent AKI, but its specificity was relatively low and, conversely, it appeared less sensitive for identifying transient AKI. In contrast, biomarkers of kidney damage, particularly NGAL, more clearly signified renal aggression, whatever the cause, likely to lead to AKI, and consequently its performance in predicting such cases was better. Thus, our results and those of others [12,37,40,41] suggest to use urinary biomarkers to better define renal involvement in COVID-19, reflecting the heterogeneity in the clinical presentation and severity and including subclinical AKI which concerned almost 20% of our cohort.

Our study had some limitations. First, our conclusions were limited by the relatively small cohort size. Despite its prospective design, urinalyses were performed at the physician's discretion, and urine samples were not collected from 14 patients. Moreover, the monocentric design of the study could limit the generalizability of the results. Second, our data were

collected during the first wave of the pandemic; consequently, our results should not be generalized to patients infected with novel SARS-CoV-2 variants [2]. Finally, interesting factors such as biomarkers of tubular function, Cystatin C (CysC) [36,42], liver-type fatty acid-binding protein (L-FABP) and  $\alpha$ 1-Microglobulin ( $\alpha$ 1M) [43] were not included in our analyses. Similarly, the combination of biomarkers with quantification of lung damage on chest CT [10] or with Doppler measurement of the renal resistive index [44,45] are promising tools that were not investigated in our study. They may help build more informative models for COVID-19-associated AKI, which is a complex phenomenon involving many mechanisms [34].

In conclusion, kidney proximal tubular dysfunction, AKI, and persistent AKI are common complications in critically ill COVID-19 patients. Disease severity, higher BMI, and use of ACEI or ARBs before ICU admission were independently associated with COVID-19-associated AKI. Comparing various urinary biomarkers, including proximal tubular dysfunction and [TIMP-2]•[IGFBP7], NGAL had the best predictive effectiveness for AKI and persistent AKI associated with COVID-19. Early detection of COVID-19-associated AKI cannot be approached in a 'one-size-fits-all' manner because of its complex pathophysiology. However, urinary NGAL levels may help physicians diagnose AKI early in combination with clinical information. Further larger multicenter studies are needed to confirm our findings.

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### **Data availability statement**

The authors consented to sharing the collected data with others. The raw data supporting the conclusions of this article will be made available by the authors without undue reservation. Data will be available immediately after the main publication and indefinitely thereafter.

## References

- [1] Wang H, Paulson KR, Pease SA, et al. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020–21. *The Lancet*. 2022;399(10334):1–13. doi: 10.1016/S0140-6736(21)02796-3.
- [2] Sullivan MK, Lees JS, Drake TM, et al. Acute kidney injury in patients hospitalized with COVID-19 from the ISARIC WHO CCP-UK study: a prospective, multicentre cohort study. *Nephrol Dial Transplant*. 2022;37(2):271–284. doi: 10.1093/ndt/gfab303.
- [3] Nadim MK, Forni LG, Mehta RL, et al. COVID-19-associated acute kidney injury: consensus report of the 25th acute disease quality initiative (ADQI) workgroup. *Nat Rev Nephrol*. 2020;16(12):765. <http://www.nature.com/articles/s41581-020-00356-5>. doi: 10.1038/s41581-020-00356-5.
- [4] Gabarre P, Dumas G, Dupont T, et al. Acute kidney injury in critically ill patients with COVID-19. *Intensive Care Med*. 2020;46(7):1339–1348. doi: 10.1007/s00134-020-06153-9.
- [5] Aroca-Martínez G, Avendaño-Echavez L, Garcia C, et al. Renal tubular dysfunction in COVID-19 patients. *Ir J Med Sci*. 2022;192(2):923–927. doi: 10.1007/s11845-022-02993-0.
- [6] Werion A, Belkhir L, Perrot M, et al. SARS-CoV-2 causes a specific dysfunction of the kidney proximal tubule. *Kidney Int*. 2020;98(5):1296–1307. doi: 10.1016/j.kint.2020.07.019.
- [7] Kormann R, Jacquot A, Alla A, et al. Coronavirus disease 2019: acute fanconi syndrome precedes acute kidney injury. *Clin Kidney J*. 2020;13(3):362–370. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7314200/>. [8] Zhou H, Zhang Z, Dobrinina M, et al. Urinalysis, but not blood biochemistry, detects the early renal impairment in patients with COVID-19. *Diagnostics*. 2022;12(3):602. doi: 10.3390/diagnostics12030602.
- [9] Xu K, Shang N, Levitman A, et al. Elevated neutrophil gelatinase-associated lipocalin is associated with the severity of kidney injury and poor prognosis of patients with COVID-19. *Kidney Int Rep*. 2021;6(12):2979–2992. doi: 10.1016/j.ekir.2021.09.005.
- [10] He L, Zhang Q, Li Z, et al. Incorporation of urinary neutrophil gelatinase-associated lipocalin and computed tomography quantification to predict acute kidney injury and in-hospital death in COVID-19 patients. *Kidney Dis*. 2021;7(2):120–130. doi: 10.1159/000511403.
- [11] Menez S, Moledina DG, Thiessen-Philbrook H, et al. Prognostic significance of urinary biomarkers in patients hospitalized with COVID-19. *Am J Kidney Dis*. 2022;79(2):257–267.e1. doi: 10.1053/j.ajkd.2021.09.008.
- [12] Casas-Aparicio G, Alvarado-de la Barrera C, Escamilla-Illescas D, et al. Role of urinary kidney stress biomarkers for early recognition of subclinical acute kidney injury in critically ill COVID-19 patients. *Biomolecules*. 2022;12(2):275. doi: 10.3390/biom12020275.



- [13] Husain-Syed F, Wilhelm J, Kassoumeh S, et al. Acute kidney injury and urinary biomarkers in hospitalized patients with coronavirus disease-2019. *Nephrol Dial Transplant*. 2020;35(7):1271–1274. doi: 10.1093/ndt/gfaa162.
- [14] Kidney Disease: Improving Global Outcomes (KDIGO). KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl*. 2013;3:1–150. doi: 10.1038/kisup.2012.73.
- [15] Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373–383. doi: 10.1016/0021-9681(87)90171-8.
- [16] Le Gall J-R, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American Multicenter Study. *JAMA*. 1993;270(24):2957–2963. doi: 10.1001/jama.270.24.2957.
- [17] The ARDS Definition Task Force. Acute respiratory distress syndrome: the Berlin definition. *JAMA*. 2012;307:2526–2533.
- [18] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604–612. doi: 10.7326/0003-4819-150-9-200905050-00006.
- [19] Payne RB. Renal tubular reabsorption of phosphate (TmP/GFR): indications and interpretation. *Ann Clin Biochem*. 1998;35(2):201–206. doi: 10.1177/000456329803500203.
- [20] Portman RJ, Kissane JM, Robson AM. Use of beta 2 microglobulin to diagnose tubulointerstitial renal lesions in children. *Kidney Int*. 1986;30(1):91–98. doi: 10.1038/ki.1986.156.
- [21] Bijvoet OL, Majoor CL. The renal tubular reabsorption of phosphate in thyrotoxicosis. *Clin Chim Acta*. 1965;11(2):181–183. doi: 10.1016/0009-8981(65)90213-5.
- [22] Work Group KA. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl*. 2012;2:1.
- [23] Chawla LS, Bellomo R, Bihorac A, et al. Acute kidney disease and renal recovery: consensus report of the acute disease quality initiative (ADQI) 16 workgroup. *Nat Rev Nephrol*. 2017;13(4):241–257. doi: 10.1038/nr-neph.2017.2.
- [24] Mohamed MMB, Velez JCQ. Proteinuria in COVID-19. *Clin Kidney J*. 2021;14(Suppl 1):i40–i47. doi: 10.1093/ckj/sfab036.
- [25] Chaudhri I, Moffitt R, Taub E, et al. Association of proteinuria and hematuria with acute kidney injury and mortality in hospitalized patients with COVID-19. *Kidney Blood Press Res*. 2020;45(6):1018–1032. doi: 10.1159/000511946.
- [26] Chan L, Chaudhary K, Saha A, et al. AKI in hospitalized patients with COVID-19. *J Am Soc Nephrol*. 2021;32(1):151–160. doi: 10.1681/ASN.2020050615.

- [27] Flammia RS, Tufano A, Proietti F, et al. Renal surgery for kidney cancer: is preoperative proteinuria a predictor of functional and survival outcomes after surgery? A systematic review of the literature. *Minerva Urol Nephrol.* 2022;74(3):255–264. doi: 10.23736/S2724-6051.21.04308-1.
- [28] Reynes J, Cournil A, Peyriere H, et al. Tubular and glomerular proteinuria in HIV-infected adults with estimated glomerular filtration rate  $\geq 60$  ml/min per 1.73 m<sup>2</sup>. *AIDS.* 2013;27(8):1295–1302. doi: 10.1097/QAD.0b013e32835fac51.
- [29] Kellum JA. Diagnostic criteria for acute kidney injury: present and future. *Crit Care Clin.* 2015;31(4):621–632. doi: 10.1016/j.ccc.2015.06.001.
- [30] Bezerra GF, Meneses GC, Albuquerque PL, et al. Urinary tubular biomarkers as predictors of death in critically ill patients with COVID-19. *Biomark Med.* 2022;16(9):681–692. doi: 10.2217/bmm-2021-0631.
- [31] Chetboun M, Raverdy V, Labreuche J, et al. BMI and pneumonia outcomes in critically ill COVID-19 patients: an international multicenter study. *Obesity.* 2021;29(9):1477–1486. doi: 10.1002/oby.23223.
- [32] Cai X, Wu G, Zhang J, et al. Risk factors for acute kidney injury in adult patients with COVID-19: a systematic review and meta-analysis. *Front Med.* 2021;8:719472. doi: 10.3389/fmed.2021.719472.
- [33] Oussalah A, Gleye S, Clerc Urmes I, et al. Long-term ACE inhibitor/ARB use is associated with severe renal dysfunction and acute kidney injury in patients with severe COVID-19: results from a referral center cohort in the northeast of France. *Clin Infect Dis.* 2020;71:2447–2456.
- [34] Redant S, De Bels D, Honoré PM. Severe acute respiratory syndrome coronavirus-2-associated acute kidney injury: a narrative review focused upon pathophysiology. *Crit Care Med.* 2021;49(5):e533–e540. doi: 10.1097/CCM.0000000000004889.
- [35] Ottolina D, Zazzeron L, Trevisi L, et al. Acute kidney injury (AKI) in patients with covid-19 infection is associated with ventilatory management with elevated positive end-expiratory pressure (PEEP). *J Nephrol.* 2022;35(1):99–111. doi: 10.1007/s40620-021-01100-3.
- [36] Serwin N, Cecerska-Heryć E, Pius-Sadowska E, et al. Renal and inflammation markers—renalase, cystatin C, and NGAL levels in asymptomatic and symptomatic SARS-CoV-2 infection in a one-month follow-up study. *Diagnostics.* 2022;12(1):108. doi: 10.3390/diagnostics12010108.
- [37] Saygili S, Canpolat N, Cicek RY, et al. Clinical and sub-clinical acute kidney injury in children with mild-to-moderate COVID-19. *Pediatr Res.* 2023;93(3):654–660. doi: 10.1038/s41390-022-02124-6.

- [38] Kerget B, Kerget F, Aksakal A, et al. Evaluation of the relationship between KIM-1 and suPAR levels and clinical severity in COVID-19 patients: a different perspective on suPAR. *J Med Virol.* 2021;93(9):5568–5573. doi: 10.1002/jmv.27099.
- [39] Luther T, Bülow-Anderberg S, Larsson A, et al. COVID-19 patients in intensive care develop predominantly oliguric acute kidney injury. *Acta Anaesthesiol Scand.* 2021;65(3):364–372. doi: 10.1111/aas.13746.
- [40] Silva-Aguiar RP, Teixeira DE, Peres RAS, et al. Subclinical acute kidney injury in COVID-19: possible mechanisms and future perspectives. *Int J Mol Sci.* 2022;23(22):14193. doi: 10.3390/ijms232214193.
- [41] Sun D-Q, Wang T-Y, Zheng KI, et al. Subclinical acute kidney injury in COVID-19 patients: a retrospective cohort study. *Nephron.* 2020;144(7):347–350. doi: 10.1159/000508502.
- [42] Pode Shakked N, de Oliveira MHS, Cheruiyot I, et al. Early prediction of COVID-19-associated acute kidney injury: are serum NGAL and serum cystatin C levels better than serum creatinine? *Clin Biochem.* 2022;102:1–8. doi: 10.1016/j.clinbiochem.2022.01.006.
- [43] Su L, Zhang J, Peng Z. The role of kidney injury bio-markers in COVID-19. *Ren Fail.* 2022;44:1280–1288.
- [44] Cruz EG, Broca Garcia BE, Sandoval DM, et al. Renal resistive index as a predictor of acute kidney injury and mortality in COVID-19 critically ill patients. *Blood Purif.* 2022;51(4):309–316. doi: 10.1159/000517469.
- [45] Garnier F, Daubin D, Larcher R, et al. Reversibility of acute kidney injury in medical ICU patients: predictability performance of urinary tissue inhibitor of metalloproteinase-2 x insulin-like growth factor-binding protein 7 and renal resistive index. *Crit Care Med.* 2020;48(4):e277–e284. doi: 10.1097/CCM.0000000000004218.

### Appendix A: Acute kidney injury in critical COVID-19 patients: usefulness of urinary biomarkers and kidney proximal tubulopathy

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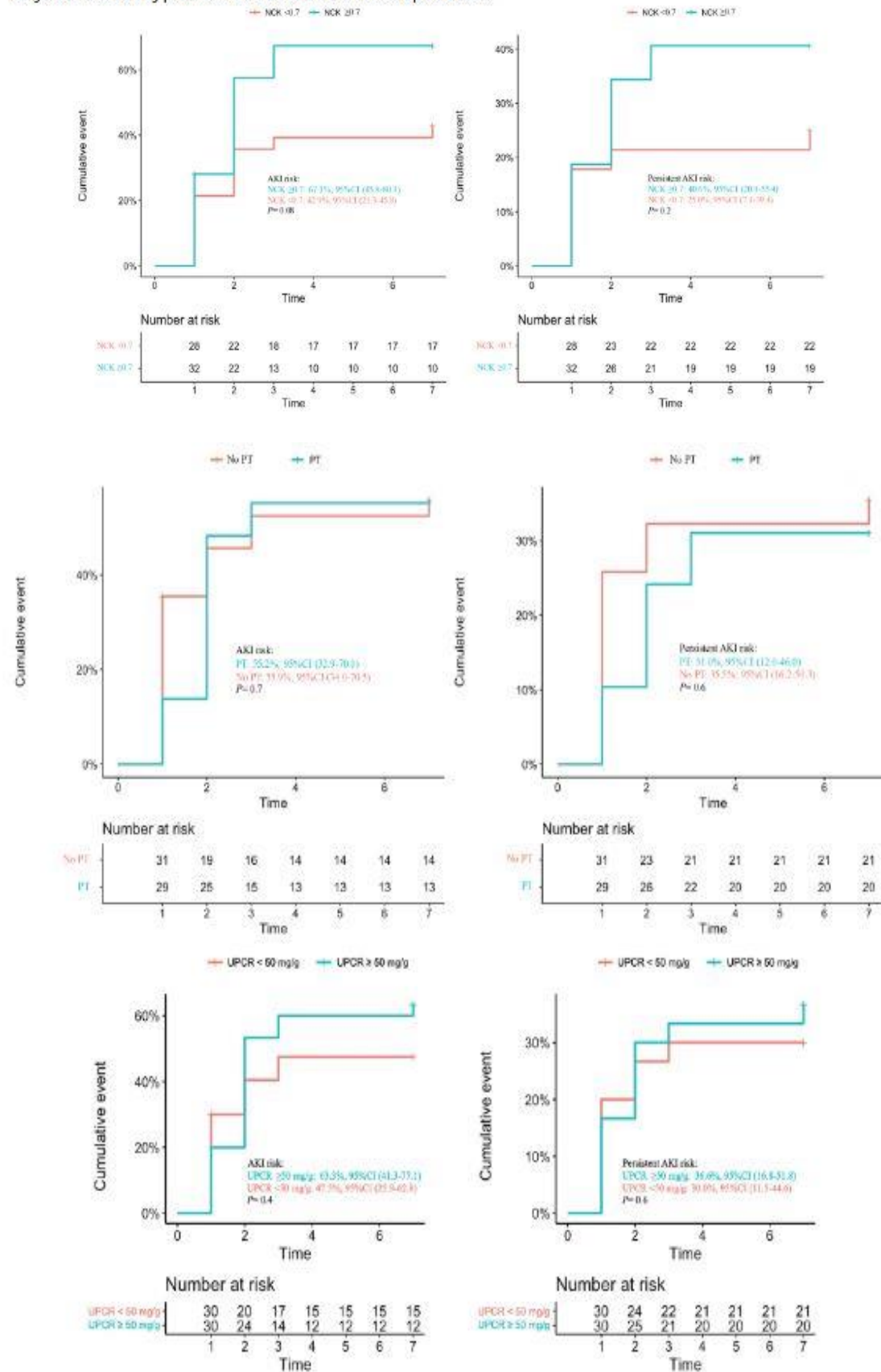
Appendix A1. Features of kidney proximal tubule dysfunction in patients with and without acute kidney injury (AKI)

Characteristic	No AKI, N=27 <sup>a</sup>	AKI, N=33 <sup>a</sup>	Overall, N=60 <sup>a</sup>	p-Value <sup>b</sup>
UPCR >0.2g/g with UAPR <0.5	1 (3.7%)	3 (9.1%)	4 (6.7%)	0.6
Aminoaciduria	13 (48%)	12 (36%)	25 (42%)	0.4
Uβ <sub>2</sub> M >0.30mg/L	2 (7.4%)	6 (18%)	8 (13%)	0.3
Hypouricemia with inappropriate uricosuria	6 (22%)	2 (6.1%)	8 (13%)	0.12
Hypophosphatemia with inappropriate phosphaturia	8 (30%)	7 (21%)	15 (25%)	0.5
Normoglycemic glucosuria	14 (52%)	20 (61%)	34 (57%)	0.5

<sup>a</sup>n (%); Median (IQR); <sup>b</sup>Pearson's Chi-squared test; Fisher's exact test; Wilcoxon rank sum test.

UAPR: urinary albumin to protein ratio; UPCR: urinary protein to creatinine ratio; Uβ<sub>2</sub>M: urinary β<sub>2</sub>-microglobulin.

Appendix A2. Cumulative incidence of acute kidney injury (AKI) and persistent AKI in COVID-19 patients according to cutoff values NephroCheck® (NCK) score ( $[TIMP-2]-[IGFBP7]/1000$ ), presence or absence of proximal tubulopathy (PT) and cutoff value of urinary protein/creatinine ratio (UPCR). IGFBP7: insulin like growth factor binding protein 7. TIMP2: tissue inhibitor of metalloproteinases 2

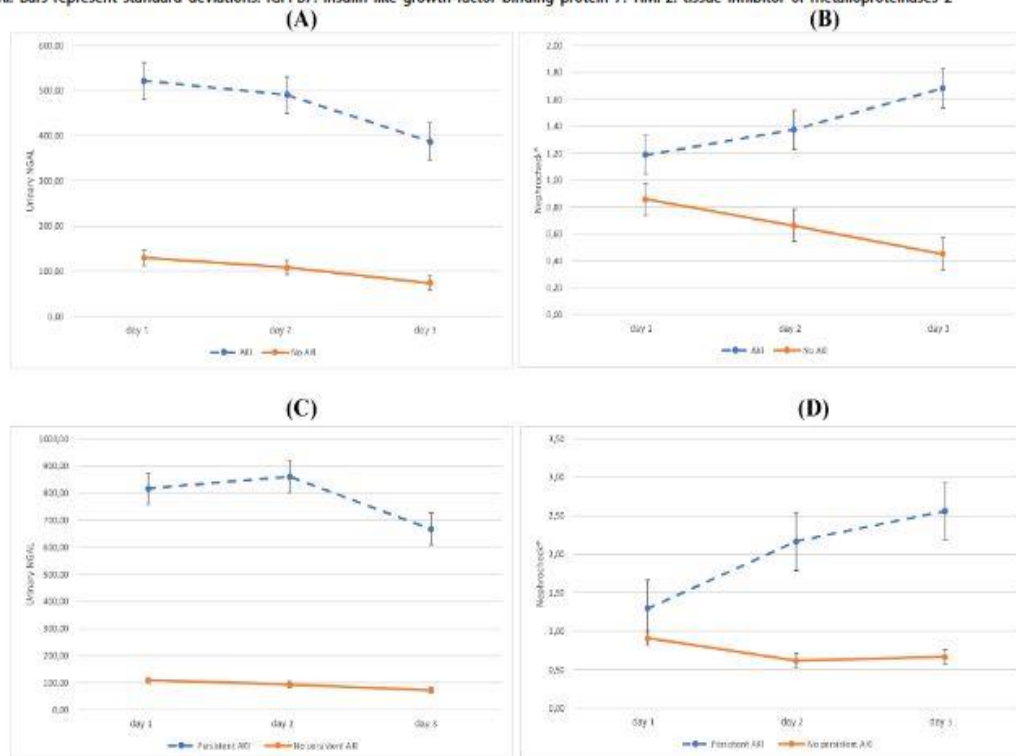


Appendix A3. Diagnostic effectiveness of urinary protein/creatinine ratio (UPCR) for acute kidney injury (AKI) and persistent AKI in critical COVID-19 patients

Characteristic	Se (%)	Sp (%)	PPV (%)	NPV (%)	AUC	95% CI
<b>AKI</b>						
UPCR ( $\geq 50$ mg/g)	74.1	57.6	58.8	73.1	0.635	0.491–0.779
<b>Persistent AKI</b>						
UPCR ( $\geq 50$ mg/g)	90.0	30.0	39.1	85.7	0.598	0.442–0.753

95% CI: confidence interval at 95%; AUC: area under the receiver operating curve; NPV: negative predictive value; PPV: positive predictive value; UPCR: proteinuria/creatininuria ratio.

Appendix A4. Kinetics of (A) mean urinary neutrophil gelatinase-associated lipocalin (NGAL) and (B) NephroCheck® (NCK) score ([TIMP-2]-[IGFBP7]/1000) from day 1 to day 3 after ICU admission in COVID-19 patients with (blue dashed line) and without (orange line) acute kidney injury (AKI) and kinetics of (C) urinary NGAL and (D) NCK from day 1 to day 3 after ICU admission in COVID-19 patients with (blue dashed line) and without (orange line) persistent AKI. Bars represent standard deviations. IGFBP7: Insulin like growth factor binding protein 7. TIMP2: tissue inhibitor of metalloproteinases 2



Appendix A5. Differences in kinetics of biomarkers in COVID-19 patients with and without acute kidney injury (AKI) and persistent AKI

Characteristic	No AKI, N=27 <sup>a</sup>	AKI, N=33 <sup>a</sup>	Overall, N=60 <sup>a</sup>	p-Value <sup>b</sup>
[TIMP-2]-[IGFBP7] at day 1	0.51 (0.27, 1.21)	0.84 (0.35, 1.78)	0.73 (0.32, 1.59)	0.2
[TIMP-2]-[IGFBP7] at day 2	0.47 (0.29, 0.85)	0.67 (0.25, 1.27)	0.57 (0.26, 1.16)	0.6
Unknown	19	13	32	
[TIMP-2]-[IGFBP7] at day 3	0.45 (0.45, 0.45)	0.92 (0.53, 1.75)	0.86 (0.47, 1.65)	0.6
Unknown	26	16	42	
NGAL at day 1	50 (50, 64)	67 (50, 117)	56 (50, 88)	0.094
NGAL at day 2	64 (50, 122)	60 (50, 176)	60 (50, 176)	0.9
Unknown	19	13	32	
NGAL at day 3	74 (74, 74)	87 (54, 163)	84 (57, 160)	0.7
Unknown	26	16	42	

<sup>a</sup>Median (IQR); <sup>b</sup>Wilcoxon rank sum test; Wilcoxon rank sum exact test. IGFBP7: insulin like growth factor binding protein 7; NGAL: neutrophil gelatinase-associated lipocalin; TIMP-2: tissue inhibitor of metalloproteinases 2; [TIMP-2]-[IGFBP7] represent the Nephrocheck® score calculated as follow: [TIMP-2] × [IGFBP7]/1000.