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# Evaluation of urine SARS-COV-2 RT-PCR as a predictor of acute kidney injury and disease severity in patients with critical COVID-19

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## Abstract

Coronavirus disease 2019 (COVID-19) is an emerging infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which began as an outbreak in Wuhan, China and has spread rapidly across the globe. Although most infections are mild, patients with severe and critical COVID-19 infections face deterioration of respiratory function and may also have extrapulmonary manifestations, mostly affecting the kidney, digestive tract, heart, and nervous system. Here, we prospectively evaluated the presence of SARS-CoV-2 genetic material using reverse-transcription polymerase chain reaction in urine samples obtained from patients with COVID-19 receiving critical care. Among 51 included patients, we found higher serum creatinine levels, a longer hospital stay, and more frequent need for dialysis in urine-positive patients.

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These findings could suggest that, in predisposed patients, a direct viral cytopathic effect may contribute to a more severe disease phenotype.

**Keywords**
Urine, severe acute respiratory syndrome coronavirus 2, polymerase chain reaction, acute kidney injury, coronavirus disease 2019, virus testing

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## Introduction

The novel coronavirus disease 2019 (COVID-19) is an emerging infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It began as an outbreak in Wuhan, China and quickly spread across the globe. Although most infections are mild, patients with severe and critical COVID-19 infections experience deteriorating respiratory function and may also develop extrapulmonary manifestations, that mostly affect the kidney, digestive tract, heart, and nervous system.[1] Acute kidney injury (AKI) is an important complication of COVID-19 infection, with an overall incidence of 4.5% but affecting up to 36% of patients with severe disease.[2] AKI is considered a marker of disease severity and a negative prognostic factor for survival.[3]

Extrapulmonary manifestations may be explained by direct infection of cells in target organs, such as the kidney because viral dissemination may occur via the bloodstream, leading to invasion of the organ and injury to resident renal cells.[4] Other plausible mechanisms are inflammation-driven because these patients may experience a cytokine storm, with systemic inflammation-mediated injury, and they are frequently dehydrated and exposed to secondary infections in the intensive care unit (ICU).[5] The direct viral cytopathic effect hypothesis is supported by previous reports of detection of viral genetic material in blood and urine samples obtained from patients with COVID-19,[6] as well as by evidence from post-mortem studies showing the presence of viral particles in the renal tissue.[7] Moreover, renal abnormalities such as hematuria and proteinuria are commonly reported and are associated with higher mortality.[8] However, the clinical relevance of a coronavirus-induced cytopathic effect in AKI with COVID-19 infection remains unclear.

Here, we prospectively evaluated the presence of SARS-CoV-2 genetic material using reverse-transcription polymerase chain reaction (RT-PCR) in urine samples obtained from patients with COVID-19 infection admitted to the ICU of Hospital São Rafael in Salvador, Brazil. We aimed to investigate a possible association between positive results of SARS-CoV-2 RT-PCR in the urine and AKI onset, as well as abnormalities in urine sediments. Our hypothesis was that the virus would be detected more frequently in patients that develop AKI, supporting a role in direct cytopathic effects on the renal parenchyma as the underlying mechanism of AKI in patients with COVID-19.

## Methods

### *Ethics statement*

The study was conducted in accordance with the principles of the Declaration of

Helsinki and received prior approval by the Ethics Committee of São Rafael Hospital in Salvador, Brazil (CAAE 34428920.0.0000.0048). All participants provided their written informed consent.

### Patient selection

All adult patients with suspected COVID-19 pneumonia requiring oxygen supplementation and who were admitted to the ICU of São Rafael Hospital between 10 July and 16 September 2020 were screened for eligibility. Patients were included in the study if they received a diagnosis of COVID-19 pneumonia confirmed by nasopharyngeal RT-PCR and chest computed tomography (CT) scan. AKI was diagnosed according to creatinine or diuresis KDIGO (Kidney Disease: Improving Global Outcomes) criteria; the baseline creatinine was the lowest serum creatinine level in the previous 3 months, obtained from the hospital electronic database. For patients without previous serum creatinine values, a 48-hour elevation of serum creatinine above 0.3 mg/dL or the KDIGO diuresis criteria were used for diagnosis.

### Isolation of viral RNA by RT-PCR

SARS-CoV-2 RNA was detected in nasopharyngeal swab samples using quantitative real-time RT-PCR. Urine samples were stored at −80°C before nucleic acid extraction and PCR testing. Urine nucleic acid extraction was performed using NucleoSpin RNA for RNA purification with an elution volume of 50 µL (Macherey-Nagel, Düren, Germany). Real-time RT-PCR was performed using the Allplex™ 2019-nCoV Assay (Seegene, Seoul, Korea) that detects the following targets: RdRP gene and N gene specific for SARS-CoV-2, and E gene for all *Sarbecovirus* betacoronaviruses, in a single tube.

For all targets, detection was considered to be a cycle threshold cut-off value ≤40 cycles and an auto-interpretation result with amplification of at least two targets. The limit of detection was 100 RNA copies/reaction. Thermal cycling conditions were according to the manufacturer's instructions, as follows: 50°C for 20 minutes, 95°C for 15 minutes, 45 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 10 s, using the AB 7500 Fast (Thermo Scientific, Waltham, MA, USA).

### Data analysis

Categorical variables were compared using the Fisher's exact test. Continuous data are presented as median and interquartile range; the Mann–Whitney U test was used for comparisons. $P$ values $<0.05$ were considered significant. Data were analyzed using the PSPP® statistical package, version 1.2.0.

## Results

Fifty-one patients were enrolled in this study and urine samples were collected from 49 patients. The entire cohort had a median age of 62 years; 62.7% of them were men, 33% had diabetes mellitus, and 60.7% had hypertension. Obesity was reported in seven patients; 35.3% of all patients required mechanical ventilation, and 29.4% used vasoactive drugs (Table 1). Prospective follow-up of the participants revealed an AKI incidence of 50.9% (26 patients); the overall mortality rate was 17.6%. AKI onset occurred during the first days after admission to the ICU or at study enrollment in 22 patients (84.6%). According to creatinine criteria, AKI occurred in 20 patients (76.9%); the remaining 6 (23%) patients were diagnosed according to KDIGO diuresis criteria. Most patients (88.2%) had serum creatinine <1.6 mg/dL on admission. RT-PCR analysis of urine samples yielded a positive result in six patients (12.2%); no correlation was found between AKI and viral RNA detection in the urine with RT-PCR.

**Table 1.** Clinical and laboratory variables of patients with COVID-19 according to urine RT-PCR.

| Variables | All (n = 51) | Urine RT-PCR positive (n = 6) | Negative urine RT-PCR (n = 45) | *P* value |
|---|---|---|---|---|
| Age (years) | 62 | 59.5 | 63.9 | 0.26* |
| Male sex | 62.7% | 100% | 57.7% | 0.05** |
| T2DM (%) | 33.3% | 83.3% | 26.6% | 0.01** |
| Hypertension (%) | 60.7% | 100% | 55.6% | 0.04** |
| CT >50% | 33.3% | 16% | 35% | 0.33** |
| VAD | 29.4% | 33% | 29% | 0.57** |
| MV | 35.3% | 50% | 33% | 0.35** |
| AKI | 50.9% | 66.6% | 48.9% | 0.35** |
| KDIGO1 | 25.5% | 16.7% | 26.7% | |
| KDIGO2 | 7.8% | 16.7% | 6.7% | |
| KDIGO3 | 17.6% | 33.3% | 15.6% | |
| Dialysis | 17.6% | 33.3% | 15.5% | 0.28** |
| Death | 17.6% | 16.7% | 17.8% | 0.71** |
| Length of hospital stay (days) | 13.5 | 19 | 13 | 0.27* |
| Illness day at the time of urine collection | 9 | 7.5 | 9 | 0.36* |
| Ferritin, mg/dL | 574 | 823 | 474 | 0.33* |
| Creatinine at admission, mg/dL | 0.88 | 1.25 | 0.81 | 0.04* |
| Creatinine, lowest level, mg/dL | 0.69 | 1.00 | 0.68 | 0.004* |
| Creatinine, highest level, mg/dL | 1.11 | 2.29 | 1.02 | 0.04* |
| Creatinine at collection timepoint, mg/dL | 0.88 | 1.56 | 0.80 | 0.04* |
| Proteinuria, positive | 49% | 66.6% | 46.6% | 0.70** |
| Hematuria, positive | 31.3% | 33.3% | 31.1% | 0.93** |

Note: Continuous data are presented as median.
COVID-19, coronavirus disease 2019; RT-PCR, reverse-transcription polymerase chain reaction; T2DM, type 2 diabetes mellitus; MV, mechanical ventilation; AKI, acute kidney injury; VAD, vasoactive drugs; CT, chest tomography with lung infiltrates; KDIGO, Kidney Disease: Improving Global Outcomes.
**Fisher's exact test, one-tailed.
*Mann–Whitney U test.

Patients' clinical and laboratory data are shown in Table 1. We found a statistically significant difference on comparing the frequency of comorbidities (hypertension, $P$=0.04; diabetes mellitus, $P$=0.01) in the group with positive RT-PCR results in urine samples as compared with the group with negative results. Patients with positive SARS-CoV-2 RT-PCR results in the urine also had significantly higher creatinine levels at admission ($P$=0.04) and had the highest creatinine values during their hospital stay. Additionally, these patients had an increased need for dialysis, with no difference in mortality compared with the group that had no virus detected in the urine (Table 1). In patients who were urine positive, proteinuria (66.6% vs. 46.6%) and the incidence of AKI (66.6% vs. 48.9%) were found to be more common. A second urine sample was collected in three patients and the results were negative in all three. Patients presenting positive RT-PCR results in urine samples also had a longer hospital stay.

## Discussion

In this prospective cohort study, we found that a positive RT-PCR result in urine samples was an infrequent finding. Compared

with patients who were urine negative, those with positive results were all male patients and had a higher prevalence of diabetes and high blood pressure, which are risk factors for severe disease. Patients who were RT-PCR-positive also had more frequent urine proteinuria, higher serum creatinine levels, and a higher prevalence of AKI, in accordance with previous reports.[2] Taken together, these findings suggest a possible cytopathic viral effect influencing the severity of renal disease.

The duration of symptoms was shorter in the group with SARS-CoV-2 detected in urine. To the best of our knowledge, another study reported a lower detection of viral RNA in the urine, but the median number of days that patients had symptoms prior to collection was 14 days.[9] We therefore speculate that viremia, as well as renal cell infection and associated viral shedding in the urine, could be early events in COVID-19 infection.

Our study findings also point to multifactorial causes already described in AKI related to COVID-19, such as exposure to nephrotoxins, volemic state, systemic inflammation, and endothelial lesions (microthrombi formation).[10] In this regard, some authors have suggested that AKI in COVID-19 is similar to sepsis-induced AKI in which most kidney lesions result from indirect hemodynamic and immunologic effects.[11] This hypothesis could explain the relatively low prevalence of proteinuria in patients who were urine negative. Additionally, only one-third of all patients had hematuria; this finding also does not support a role in direct viral-mediated cytopathic effects in the pathophysiology of AKI for a high proportion of patients with COVID-19.

Our study has some limitations. This was a single-center, observational study with a limited number of participants. We also could not successfully obtain follow-up urine specimens as most patients with AKI already had AKI upon enrollment in the study.

In summary, we prospectively examined urine samples obtained from patients with COVID-19 for SARS-CoV-2 RNA and found relatively low detection of viral RNA, which does not support a relevant role in cytopathic viral effects for most patients with AKI. However, the subgroup of patients who tested positive for SARS-CoV-2 in urine samples presented a higher frequency of diabetes and hypertension. In this subgroup, we identified higher serum creatinine levels, a longer hospital stay, and a more frequent need for dialysis. Although preliminary, these findings lead to speculation that in predisposed patients, a direct viral cytopathic effect could contribute to a more severe disease phenotype.

## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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