High SARS-CoV-2 Viral Load in Urine Sediment Correlates with Acute Kidney Injury and Poor COVID-19 Outcome

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**Abstract:**

**<b>Background</b>**

Acute kidney injury (AKI) is a complication of coronavirus disease 2019 (COVID-19) that is associated with high mortality. Despite documented kidney tropism of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there are no consistent reports of viral detection in urine or correlation with AKI or COVID-19 severity. Here we hypothesize that quantification of SARS-CoV-2 viral load in urine sediment from COVID-19 patients correlates with occurrence of AKI and mortality.

**<b>Methods</b>**

SARS-CoV-2 viral load in urine sediments (U-viral load) was quantified by qRT-PCR in 52 patients with PCR-confirmed COVID-19 diagnosis, hospitalized between March 15th and June 8th, 2020. Immunolabeling of SARS-CoV-2 proteins Spike and Nucleocapsid was performed in two COVID-19 kidney biopsies and urine sediments. Viral infectivity assays were performed from 32 urine sediments.

**<b>Results</b>**

Twenty COVID-19 patients (39%) had detectable SARS-CoV-2 U-viral load, of which 17 (85%) developed AKI with an average U-viral load 4-times higher than non-AKI COVID-19 patients. U-viral load was highest (7.7-fold) within two weeks after AKI diagnosis. A higher U-viral load correlated with mortality but not with albuminuria or AKI stage. SARS-CoV-2 proteins partially colocalized with the viral receptor ACE2 in kidney biopsies in tubules and parietal cells, and in urine sediment cells. Infective SARS-CoV-2 was not detected in urine sediments.
<b>Conclusion</b>
Our results further support SARS-CoV-2 kidney tropism. A higher SARS-CoV-2 viral load in urine sediments from COVID-19 patients correlated with increased incidence of AKI and mortality. Urinary viral detection could inform medical care of COVID-19 patients with kidney injury to improve prognosis.
Significance statement

Acute kidney injury (AKI) has been recognized as a common complication of COVID-19 and associated with disease severity and mortality. The mechanisms behind these associations remain obscure, in part due to unsuccessful attempts to consistently detect the novel coronavirus SARS-CoV-2 in urine, despite evidence of kidney tropism. This study consistently quantifies SARS-CoV-2 genome via qRT-PCR in cells of urine sediments from COVID-19 patients. It was found that viral load in urine sediment was higher within two weeks of the AKI event among COVID-19 patients, and it correlated with increased risk of death. Quantification of viral load in urine sediment offers a non-invasive approach that could help identify and care for COVID-19 patients at higher risk of kidney injury and poor outcome.
High SARS-CoV-2 viral load in urine sediment correlates with acute kidney injury and poor COVID-19 outcome

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Abstract

Background

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Methods

SARS-CoV-2 viral load in urine sediments (U-viral load) was quantified by qRT-PCR in 52 patients with PCR-confirmed COVID-19 diagnosis, hospitalized between March 15th and June 8th, 2020. Immunolabeling of SARS-CoV-2 proteins Spike and Nucleocapsid was performed in two COVID-19 kidney biopsies and urine sediments. Viral infectivity assays were performed from 32 urine sediments.

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Twenty COVID-19 patients (39%) had detectable SARS-CoV-2 U-viral load, of which 17 (85%) developed AKI with an average U-viral load 4-times higher than non-AKI COVID-19 patients. U-viral load was highest (7.7-fold) within two weeks after AKI diagnosis. A higher U-viral load correlated with mortality but not with albuminuria or AKI stage. SARS-CoV-2 proteins partially colocalized with the viral receptor ACE2 in kidney biopsies in tubules and parietal cells, and in urine sediment cells. Infective SARS-CoV-2 was not detected in urine sediments.

Conclusion

Our results further support SARS-CoV-2 kidney tropism. A higher SARS-CoV-2 viral load in urine sediments from COVID-19 patients correlated with increased incidence of AKI and mortality. Urinary viral detection could inform medical care of COVID-19 patients with kidney injury to improve prognosis.
Introduction

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and the associated coronavirus disease 2019 (COVID-19) rapidly became a pandemic. Severe COVID-19 cases present with dyspnea and hypoxemia, leading to respiratory failure. However, SARS-CoV-2 does not exclusively infect respiratory tract cells, as documented in recent studies in enterocytes, endothelial and kidney cells. It is not clear to what extent this multiorgan tropism contributes to poor COVID-19 outcome, mainly due to lack of conclusive studies addressing viral presence in target organs in relation with COVID-19 complications. One of the most common complications of COVID-19 is acute kidney injury (AKI), which occurs in up to 49% of patients, with higher rates in minority groups, and is linked to poor COVID-19 prognosis and mortality. Despite the documented presence of SARS-CoV-2 in kidney tissue, efforts to detect viral genetic material or infective particles in urine have been inconclusive. Most studies reported absent or a very low detection rate of viral genetic material in whole urine, and only few laboratories have examined the presence of infective SARS-CoV-2. This lack of in-depth urine analysis has prevented from establishing an association between urine SARS-CoV-2 load and kidney injury, precluding from taking advantage of an easily accessible non-invasive sample as diagnostic tool for COVID-19 patients. It is unclear why virus detection in urine is so challenging. Possible reasons could be related to variability in urine volume, incomplete replication or release by infected kidney cells, or virus destruction in urine by excreted proteases and RNAses. All these factors could be minimized by detection methodologies of increased sensitivity aimed to measure SARS-CoV-2 presence directly in cells of renal origin, like cells shed in urine sediments. Here we tested the hypothesis that SARS-CoV-2 viral load (U-viral load) can be quantified in urine sediment and it correlates with occurrence of AKI and risk of death among COVID-19 patients. A better understanding of the impact of SARS-CoV-2 presence in the kidney, its detection in urine and relationship to kidney damage could better inform medical decisions to treat COVID-19 patients.

Methods

Patients

During the time frame of our study, 1320 patients were admitted to the Henry Ford Hospital in Detroit, Michigan, with a clinical diagnosis of COVID-19. Between March 15th and June 8th 2020, a cohort of 652 patients enrolled...
in the Translational and Clinical Research Center biobank at the Henry Ford Hospital. From our biobank, 52 patients were enrolled with a COVID-19 diagnosis confirmed by laboratory RT-PCR in nasopharyngeal swabs or tracheal aspirates. Clinical history, course of COVID-19 and diagnosis of AKI based on the clinical chart were abstracted from the electronic medical record. Subsequently, the presence of AKI, its KDIGO stage, and the presence of renal replacement therapy was adjudicated by two independent and blinded nephrologists. Any discrepancy was reconciled by a third one. The results of the adjudication were used for all analyses reported (Figure 1). The institutional review board at Henry Ford Health System approved specimen collection and biobanking. Each patient, or their legal representative provided informed consent prior to participation and only deidentified data was analyzed. Urine specimens were collected from 14 consented healthy control volunteers, 18 COVID-19 patients with no AKI, and 33 COVID-19 patients in the following weeks after an AKI episode (up to 8 weeks). Only one patient in the cohort was not available for specimen collection after the AKI episode and was excluded from the qRT-PCR analysis.

**Urine sediment specimen collection**

Urine samples were obtained as spontaneously voided or bladder-catheterized specimens, and only included in the study if fresh urine sample could be obtained and processed immediately by study staff. Urine sediments were obtained within 2-4 h of sampling by centrifugation at 1000x \( g \) for 10 minutes, followed by two washes with cold PBS buffer. All samples were handled in a Biosafety level-2 laboratory following approval from internal IRDBC. Viral RNA was extracted by resuspending urine sediments in lysis buffer from the viral RNA extraction kit (Takara Bio USA, Ann Arbor, MI), following the manufacturer’s instructions. RNA was quantified in a Qubit 4 fluorometer with the Qubit RNA High Sensitivity Assay Kit (Thermo Fisher Scientific, Waltham, MA). To estimate RNA sample purity, the A260/A280 ratios were measured by nanodrop (Gen5 Synergy H1 plate reader, BioTek, VT) in separate aliquots of ten samples of the highest RNA concentration, and determined to be on average 2.3 ± 0.3.

**Real-time RT-PCR for SARS-CoV-2 in urine sediments**

Viral RNA was extracted from urine sediments with a commercial kit (Takara Bio USA, Ann Arbor, MI). Between 10-500 ng of RNA were reverse-transcribed using SuperScript III reverse transcriptase (Thermo Fisher Scientific) and random hexamers. 5 \( \mu L \) of the resulting cDNA reaction were used for real-time PCR in a ViiA 7 Real-Time
PCR System (Applied Biosystems, Foster City, CA). The cDNA was diluted 1:10, 1:20 or 1:50 to assure the measurements were within the linear range of the standard curve (Supplemental figure S1). Oligonucleotide primers specific for the SARS Cov-2 Spike (S) and Nucleocapsid (N) genes were previously developed. The S primer set\textsuperscript{22} had the following sequences: Forward 5‘-3‘ CAATGGTTTAACAGGCACAGG; Reverse 5‘-3‘ CTCAAGTGTCTGTGGATCACG, and N primers correspond to the N2 set developed by the Centers for Disease Control (CDC) (https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf). All primers were synthetized by Eurofins Genomics (Louisville, KY). Real-time SYBR-green PCR mix was from Bio-Rad (Hercules, CA). Urine sediments from 14 healthy non-symptomatic controls, with no history of COVID-19 or AKI, were analyzed to establish background RT-PCR signal. Viral RNA copies were quantified with a standard curve of heat-inactivated SARS-CoV-2 complete genome (BEI resources, NIAID, NIH, Manassas, VA. Cat no. NR-52347, Lot no. 70033926). The number of viral copies /ng RNA was calculated as:

\[ \text{Viral copies} \times \text{PCR dilution factor} / \text{ng RNA}. \]

The intra- and inter-assay coefficients of variation were 0.9 ± 0.1% and 1.8 ± 0.3% respectively as determined by triplicates run in parallel and sequentially. Single-band amplification was verified by gel electrophoresis.

**SARS-CoV-2 infectivity assay**

Virus infectivity was assessed at a Biosafety level-3 facility at Michigan State University Veterinary Diagnostic Laboratory. African green monkey Vero E6 cells (ATCC, Manassas, VA) were grown at 80% confluency in medium 199 with Earle’s balanced salts (Millipore Sigma, St. Louis, MO) supplemented to 6% U.S. origin fetal bovine serum (Millipore Sigma, St. Louis, MO). Urine sediment lysate was brought to 1 ml with Bovarnick’s buffer (pH 7.0) then centrifuged at 8,000 x g for 5 minutes to pellet debris. The clarified liquid was passed through a 0.22 μm syringe filter directly onto cell cultures. Cells were cultured and monitored daily for cytopathic effect for up to 10 days. At the end of this period, RNA was extracted and real-time qRT-PCR performed for SARS-CoV-2 genes N and S to confirm presence or absence of viral replication.

**Immunofluorescence and confocal microscopy**

Kidney biopsy sample slides were obtained from two COVID-19, RT-PCR-confirmed patients, and two COVID-19 negative patients (negative controls) by the Translational and Clinical Research Center. Biopsies were indicated for unexplained nephrotic range proteinuria with clinical concern for rapidly progressing
glomerulonephritis in COVID-19 patients. Formalin fixed Paraffin-Embedded slices were first de-paraffinized with xylene and then rehydrated gradually through 100% ethanol to distilled water. Slides were incubated for 10 min with 0.05% Triton X-100 at room temperature (RT) for permeabilization followed by antigen retrieval with citric Acid buffer (10mM, pH 6.0). Slides were then blocked with 5% BSA to block nonspecific binding. Slides were incubated with primary antibody 1:200 dilution of SARS-CoV-2 Spike monoclonal antibody (clone 1A9, Gentex, Irvine, CA) or SARS-CoV-2 nucleocapsid antibody (clone 6H3, Gentex, Irvine, CA) at 4°C overnight. This was followed by 1:100 of Alexa-Fluor 647 donkey anti-mouse IgG (Molecular probes, Eugene, OR) for 1 hour at room temperature. To label ACE2, slides were then incubated with 1:50 dilution of ACE2 rabbit monoclonal antibody (clone EPR4435, Abcam, Cambridge, MA) after blocking with 1% BSA, followed by incubation with 1:100 Cyanine3 (Cy3) donkey anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA) for 1 hour at RT. Finally, slides were counter-stained with DAPI at 1:2000 dilution for 5 minutes at RT. Controls for non-specific secondary antibody binding were performed without primary antibodies, and were completely negative. COVID-19 negative and COVID-19 positive slides were imaged the same day, with identical settings using a Leica Confocal/multiphoton microscopy system TCS SP8 MP (Leica Microsystems, Germany), 63X, 1.40 oil CS2 (HC PL APO) lens. We also tested antibodies against SARS-CoV nucleocapsid protein (A50, 1:250, Rockland antibodies, Limerick, PA) or SARS-CoV Membrane protein (A55, 1:200, Rockland antibodies, Limerick, PA)23. All these antibodies have recently been used by other investigators to detect SARS-CoV-2 in respiratory tissue and placenta5,24 and in human liver duct organoids25.

**Albumin/ creatinine ratio**

Spontaneously voided or bladder-catheterized urine samples were obtained from hospitalized patients and briefly centrifuged (1000xg, 10 min) to eliminate particulate material. Supernatants were used for determination of urine albumin (Abcam, Cat ab108788) and creatinine (BioAssay, Hayward CA) concentration with ELISA kits. The measured urine albumin and creatinine concentrations were used to calculate the albumin / creatinine ratios as mg/g.

**Statistical analysis**

For descriptive purposes, continuous variables were summarized by means and standard deviations, and categorical variables are summarized by frequency counts and percentages. Between group differences in
continuous variables were assessed using non-parametric Kruskal-Wallis test. For categorical variables, between group frequency differences were assessed using Fisher’s exact test. Correlations between continuous variables were tested using Spearman rank correlations. All statistical analyses were performed with GraphPad Prism 8.2.1 (GraphPad Software, San Diego, CA), with the exception of the analysis for inpatient mortality. The association between U-viral load and inpatient COVID-19 mortality was performed using the Fine and Gray competing risk model with the R statistical programming language package “cmprsk”. In this analysis, inpatient mortality was modelled as the primary hazard, and hospital discharge alive was modelled as the secondary hazard. Patients who were still in the hospital and alive as of June 8th 2020 were censored. To account for multiple admissions, the total inpatient days across all admissions for a patient was considered as the inpatient time to event. Hazard ratios (HR) and 95% confidence intervals (95%CI) for the association of U-viral load with mortality were calculated both unadjusted and adjusted for age, sex, and African American race-ethnicity. P-values less than 0.05 were considered statistically significant.

Results

SARS-CoV-2 detection in urine sediments from COVID-19 AKI patients

Baseline characteristics of our study cohort of 52 patients with confirmed COVID-19 diagnosis are summarized in Table 1. The average follow-up time from COVID-19 related admission was 53.7 ± 17.9 days. Two SARS-CoV-2 genes (Spike [S] and Nucleocapsid [N]) were successfully detected by RT-PCR and quantified by extrapolation to a standard curve of SARS-CoV-2 genome (Supplemental Figure S1). An average U-viral load of 1,730 ± 344 copies/ng RNA was measured in non-AKI COVID-19 specimens (not significant vs. background in COVID-19-negative controls, P=0.38) (Figure 2A). In specimens from COVID-19 patients that developed AKI, the U-viral load was 3.9-times higher (P=0.001) (mean: 6,779 ± 1,479 copies/ng RNA) in comparison to background in COVID-19-negative controls (Figure 2A). In patients where urine was collected within the first two weeks following an AKI episode, the U-viral load was significantly higher (13,266 ± 3,033 copies/ng RNA, P=0.004 vs. non-AKI) (Figure 2B), returning to levels comparable to non-AKI after 2 weeks (3,212 ± 897 copies/ng RNA, p>0.999 vs. non-AKI). To assess the prevalence of high U-viral titers among AKI patients, we
set a threshold of three standard deviations of the background signal from non-infected controls. Approximately half of the patients with AKI had SARS-CoV-2 U-viral loads above this threshold, compared with only 3 out of 18 (17%) of the non-AKI group (P=0.02) (Figure 2C), indicating a larger proportion of AKI patients with higher SARS-CoV-2 viral load in urine sediments. The amount of RNA purified from COVID-19 patients with or without AKI was not significantly different (Supplemental figure S2, P> 0.999) and there was no correlation between U-viral load and starting amount of RNA in RT-PCR reaction (Spearman rho correlation coefficient= 0.08, R²= 0.006), indicating that viral load in AKI patients is not artificially high due to increased cell shedding. To rule out additional possible confounding factors, we further determined that the method of urine collection (spontaneous voiding vs. catheterization), the presence of blood in urine, or requirement of mechanical ventilation were not associated with higher U-viral loads (Supplemental figure S3).

Altogether, these results indicate higher levels of SARS-CoV-2 genetic material in urine sediments from COVID-19 patients that develop AKI.

**SARS-CoV-2 load in urine and COVID-19 severity**

U-viral load was associated with COVID-19 inpatient mortality (P<0.001), with each increase of 10,000 viral copies/ng RNA associated with a 2.87-fold (95%CI 1.76-4.68) increased risk of death, and this increase in risk was largely unchanged with adjustment for age, sex and African American race-ethnicity (HR=3.28; 95%CI 1.77-6.06; P<0.001). Among deceased subjects, the U-viral load reached a high of 11,073 ± 2816 copies/ng RNA at an average of 9 days before death. This viral load was 3-times higher than the average 3,377 ± 860 copies/ng RNA (P=0.03) recorded in subjects that were still alive at time of cutoff (Figure 3A). Figure 3B displays the cumulative incidence of inpatient mortality in those patients below and above the average of the highest recorded U-viral load (5,141 copies/ng RNA) across all patients. At 60 days, those above and below this threshold had cumulative mortality rates of 62% and 9% respectively, with a corresponding hazard ratio of 11.5 (95%CI 3.27-40.30, P<0.001). Table 2 puts some clinical perspective on the subgroup of deceased patients. A large majority had developed AKI (83.3%), and only two were in the non-AKI group. As expected, the predominant cause of death were respiratory and systemic complications from COVID-19. The only non-COVID-19-related death on record was one case of metastatic cancer. This patient was in the non-AKI group and had low measurable viral
load in urine sediment (539 copies/ng RNA) suggesting that the poor outcome was not related to COVID-19. Besides the higher viral load in urine sediment associated with subsequent risk of death, we did not observe any correlation between mortality and duration of COVID-19, need of ventilation, sepsis, or changes in blood pressure (Table 2). However, since the number of deceased patients (n=12) was relatively small, the statistical power was low. Similarly, we did not observe any strong evidence of risk factors associated with patients presenting with the highest U-viral loads above 5,141 copies/ng RNA in our cohort (Supplemental table 1).

In subjects with a urine albumin / creatinine ratio (ACR) higher than 30 mg/g, the average U-viral load tended to be higher, but not significantly different (ACR < 30: 3,839 ± 1,330 viral copies/ng RNA vs. ACR ≥ 30: 5,649 ± 1,329 viral copies/ng RNA, P= 0.50) (Figure 3C). We did not find significant associations of U-viral load with serum creatinine, blood in urine, C-reactive protein (Supplemental figure S3), BUN, stage of AKI, or need for renal replacement therapy (Figure 3D, 3E). This weak correlation with renal parameters and markers of injury may be reflective of transient effects related to the time of collection.

**Presence of SARS-CoV-2 proteins in kidney tissue**

To provide additional evidence of SARS-CoV-2 infection in the kidney, we performed immunolabeling for SARS-CoV-2 proteins Spike and Nucleocapsid in kidney biopsies from COVID-19 positive and negative patients (Figure 4). Positive labeling for viral proteins was detected in different kidney cell types, including proximal tubules (Figure 4A, 4C, 4D), parietal cells (Figures 4B), and distal tubules (with the appearance and size of collecting ducts) (Supplemental Figure S4). Relatively low immunoreactivity was observed in glomerular cells (Figure 4B). Only background signal was observed in biopsies from COVID-19-negative patients (Figure 4E-G). ACE2, the receptor for viral entry, was abundant in proximal tubules and, to a lesser extent, parietal cells, and co-localized with intracellular SARS-CoV-2 in some proximal tubule cells (Figure 4A, 4B, and Supplemental Figure S4B). We also conducted immunolabeling in fresh sediments imbedded in matrigel. SARS-CoV-2 Spike was detected in single cells from a patient with positive urine RT-PCR (Figure 5A) but absent in COVID-19 negative patients (Figure 5B). ACE2 expression was detected in the same cells (Figure 5), but not all cells in urine sediment expressed ACE2. Co-expression of ACE2 and the viral protein Nucleocapsid was also observed in urine
sediments from three additional patients with urine-positive RT-PCR (Supplemental Figure S5). The origin or identity of these cells was not determined.

**Infectivity of SARS-CoV-2 material from urine**

It is unclear whether infective virions are shed in the urine, and a previous study failed to detect this. We studied 32 fresh urine sediments, 28 from COVID-19 positive patients and 4 negative controls, for cytopathic viral growth in inoculated Vero E6 cells. Two out of 28 (7.1%) urine sediment specimens generated cytopathic growth (both from AKI-diagnosed patients). However, subsequent RT-PCR failed to amplify any SARS-CoV-2 genetic material isolated from Vero E6 cells, suggesting that the cytopathic effect observed was secondary to a viral infection other than SARS-CoV-2.

**Discussion**

During the time frame of our study, 1320 patients were admitted to our institution in Detroit, Michigan, with a clinical diagnosis of COVID-19. Retrospective review of our medical records showed that 614 (46.5%) were also diagnosed with AKI. AKI was present in 172 of 225 (76.4%) deceased patients. A high incidence of AKI (up to 49% in U.S. minority groups) has been documented in COVID-19 epicenters around the world. Furthermore, the mortality rate observed among the AKI subset of COVID-19 patients is disproportionately high. To gain insight into the impact of COVID-19 in kidney dysfunction, the present study was targeted to a cohort of 52 patients with laboratory RT-PCR diagnosis of COVID-19. Here we report higher viral copy number detected in urine sediment from COVID-19 patients that developed AKI, and a strong correlation between urine sediment viral load and subsequent mortality.

Urine sediments represent a non-invasive diagnostic tool with potential for assessing COVID-19 AKI patients at risk. Previous smaller sized studies utilizing whole urine detected none or only one SARS-CoV-2-positive urine specimen. A recent larger study on 81 patients showed a SARS-CoV-2 detection rate of 7% in whole urine. In an effort to maximize detection in urine, in the present study we collected urine sediments from COVID-19 patients, and measured SARS-CoV-2 directly from cells contained in the sediment utilizing a sensitive qRT-PCR assay previously developed for respiratory samples. We report a detection rate of 39% of tested
urine sediment specimens, significantly higher than a 7.5% detection rate previously reported by a similar study in a cohort of 53 COVID-19 patients. This increased sensitivity is probably due to different RT-PCR methodologies, since this previous study utilized a probe-based RT-PCR assay for detection of SARS-CoV-2 genes N and Orf-1ab. In our hands, equivalent probe-based assays for genes N and E did not yield positive results. In comparison, the SYBR green-based assays we adopted for S and N genes showed higher sensitivity. Importantly, it has not been possible so far to use urine viral load to establish a link between AKI and COVID-19, while this association has been widely discussed in the medical literature, mostly based on case reports or autopsy findings. The quantitative approach presented here using a viral genome standard curve revealed a positive correlation between SARS-CoV-2 copy number in urine sediment, a non-invasive sample, and higher frequency of AKI and mortality. Since different methodologies result in varying sensitivity, we emphasize the advantages of standard curve-based quantitative methods for better comparisons across different studies to help identifying COVID-19 patients at higher risk from AKI.

Due to limited specimen availability, we did not thoroughly investigate the cause of AKI in our cohort. A kidney biopsy from one of our COVID-19 positive patients shows evidence of tubular and glomerular damage, which is consistent with previous reports (Supplemental Figure S6). To our knowledge, there is no consensus in the literature on how kidney complications develop in hospitalized COVID-19 patients. AKI could arise from a systemic immune response, hemodynamic alterations, hypercoagulability, viral kidney tropism or a combination of these. While these mechanisms are not mutually exclusive, the evidence for a localized immune response in kidneys of COVID-19 patients is inconsistent. Recent reports demonstrated autoimmune-like glomerular basement membrane disease, lymphocyte infiltration, arteriolar thrombosis and collapsing glomerulopathy in kidneys of COVID-19 patients. Other studies did not find strong evidence of immune infiltration in kidney tissue during SARS-CoV-2 infection. These differences may be related to underlying conditions in populations from different ethnic backgrounds. Concerted actions and establishment of a national biopsy tissue repository should be generated to address these questions.

An alternative cause of kidney damage as a consequence of COVID-19 is active SARS-CoV-2 tropism in the kidney. Recent studies reported detectable SARS-CoV-2 proteins in post-mortem kidneys, in addition to electron microscopy detection of virus-like particles. However, concerns have been raised about the
misinterpretation of electron microscopy evidence based on morphological features and their similarity to normal intracellular structures. The present study provides additional immunodetection-based evidence of expression of two SARS-CoV-2 proteins, Nucleocapsid and Spike, in kidney cells from COVID-19 biopsies (Figure 4 and S4). Sites with positive SARS-CoV-2 immunolabeling were proximal tubules, distal nephron, parietal cells and potentially inflammatory cells, as well as cells shed in the urine. Expression of ACE2 in kidney proximal tubule cells has been associated with the ability of SARS-CoV-2 to infect cultured proximal tubule cells. However, whether this results in cytotoxicity remains to be determined. A more recent study showed clinical and histological manifestations consistent with proximal tubule injury in a group of COVID-19 patients. The strong ACE2 labeling we observed in the brush border of proximal tubules in COVID-19 positive (n=2) and negative (n=2) kidney biopsies is consistent with previous literature. We observed very low ACE2 immunolabeling in cells other than proximal tubules. However, this does not preclude the expression of very low levels of ACE2, which has been detected in other kidney cells by RNA sequencing.

Despite finding SARS-CoV-2 genetic material and protein in urine sediments, viability assays for cytopathic viral growth were negative, consistent with a previous report. The reasons behind these observations are not clear and invite further investigation designed for this purpose. Some possibilities may include inability of kidney cells to assemble or release infective virions, or subsequent destruction by extracellular proteases in the urine. However, lack of infective material in urine does not preclude from the fact that SARS-CoV-2 may be able to reach and infect kidney cells as demonstrated by the detection of viral proteins in kidney biopsies in the present report, and previous studies in postmortem kidney. Absence of infectious SARS-CoV-2 particles in urine is independent of whether viral presence in urine material is associated with AKI and risk of poor COVID-19 outcome. This fact allowed us to present evidence in support of our hypothesis of direct kidney tropism, and detectable and quantifiable SARS-CoV-2 genetic material in urine sediment, providing an accessible specimen with diagnostic and predictive potential in terms of incidence of AKI and COVID-19-related mortality. Our study was not designed to determine whether SARS-CoV-2 kidney tropism directly causes AKI. This is a standing question requiring mechanistic studies in animal models or organoids directly addressing this possibility.

Limitations of the study: Our cohort was selected to study patients with kidney involvement. Thus, the elevated rate of AKI is not representative of the overall population of COVID-19 patients. Our patient population is largely...
African American (70%), with high prevalence of comorbidities like chronic kidney disease, hypertension, diabetes and high mortality (Table 1). AKI and prior chronic kidney disease diagnosis are reportedly higher in African Americans with a COVID-19 diagnosis. It is possible that our patient population is highly susceptible to developing AKI and this may not be representative of other populations. That said, we relied on adjudicated AKI diagnoses/outcomes for analyses rather than medical record diagnoses increasing the accuracy of our kidney outcomes. A larger cohort should be studied to determine the influence of ethnic background or prior chronic kidney disease diagnosis. In addition, a larger cohort can also better address the predictive value of SARS-CoV-2 RT-PCR detection in urine sediments to determine risk of AKI and severity of COVID-19. This predictive potential is also most likely tied to parallel monitoring of viral loads in urine and blood. In this regard, a previous study in the related SARS coronavirus found that viral detection in blood seems to peak transiently during the first week of infection, followed by a peak in urine and stool afterwards. Determining whether SARS-CoV-2 follows a similar dynamic and its relationship to development of AKI could provide a powerful diagnostic tool. These considerations, together with additional variables like gender and age should be addressed to inform revised guidelines for care of COVID-19 patients with kidney involvement, in efforts to identify patients at higher risk and to better allocate scarce resources.
Author contributions

PAO formulated the hypothesis. PSC and PAO designed the experiments and wrote the manuscript. PSC analyzed the data and prepared figures. GS, SLM, MNK and SS recruited patients, reviewed medical records and compiled table 1. PSC, DM and JF performed RT-PCRs. SS analyzed albumin/creatinine ratios. KU, JU, JY and YS performed the acute kidney injury adjudications, established the patient selection criteria and contributed to the outline of the manuscript. TDL and PAO performed immunofluorescences. AML performed statistical analysis and prepared inpatient mortality figure. SB performed virus infectivity assays. AHO processed the kidney biopsies. All authors contributed to the final edited version of the manuscript.

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Disclosures

K. Umanath reports Consultancy Agreements with Astra Zeneca, ICON/ Novo Nordisk; Research Funding from AstraZeneca, GlaxoSmithKline, Sentien, ReCor Medical; and Scientific Advisor or Membership with The Collaborative Study Group - Vice-President and Board of Directors. P. Ortiz reports Research Funding from NIH, NIDDK, American Heart Association; Scientific Advisor or Membership with American Journal of Physiology-Renal Physiology on Editorial Board, Past Chair AHA-Kidney in Cardiovascular Diseases Council, NIH-Study section (HM) permanent member 2013-2019, and Hypertension (journal). J. Yee reports Consultancy Agreements with AstraZeneca, GLG; Ardelyx, EBSCO/Dynamed, Bayer; Ownership Interest in Vasc-Alert(TM); Honoraria from AstraZeneca, Fresenius Medical Corporation, North America, Cara Therapeutics, Bayer, Gerson Lehman Group, AlphaSights, Ardelyx; Patents and Inventions with Vasc-Alert(TM); Scientific Advisor or Membership AstraZeneca, Ardelyx, Bayer; and Other Interests/Relationships with National Kidney Foundation, Editorial Board for Am J Kidney Dis, EBSCO/DynaMed Editorial Board, Elsevier Clinical Key Author, Elsevier Section Editor, Ferri's Clinical Advisor 2022, BMC Nephrology Editorial Board, Springer Heart Failure Reviews

The remaining authors have nothing to disclose.

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Supplemental Material Table of Contents:

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Supplemental Figure S5. Expression of Nucleocapsid and ACE2 in cells shed in urine sediment from SARS-CoV-2 urine-positive patients.

Supplemental Figure S6. Kidney histopathology of COVID-19 patient with AKI.
References


Table 1: Baseline characteristics of the study cohort.

<table>
<thead>
<tr>
<th></th>
<th>COVID-19</th>
<th>COVID-19 + AKI</th>
<th>COVID-19 - no AKI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>52 (100%)</td>
<td>34 (65.4%)</td>
<td>18 (34.6%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>62 ± 13</td>
<td>64 ± 12</td>
<td>58 ± 15</td>
<td>0.24</td>
</tr>
<tr>
<td>Gender - no. (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Male</td>
<td>30 (57.7%)</td>
<td>24 (80.0%)</td>
<td>6 (20.0%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>22 (42.3%)</td>
<td>10 (45.5%)</td>
<td>12 (54.5%)</td>
<td></td>
</tr>
<tr>
<td>Race - no. (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>African American</td>
<td>37 (71.2%)</td>
<td>23 (62.2%)</td>
<td>14 (37.8%)</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>11 (21.2%)</td>
<td>8 (72.7%)</td>
<td>3 (27.3%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (3.8%)</td>
<td>2 (100%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1 (1.9%)</td>
<td>1 (100%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1.9%)</td>
<td>0</td>
<td>1 (100%)</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.72 ± 0.11</td>
<td>1.75 ± 0.09</td>
<td>1.67 ± 0.09</td>
<td>0.008</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94.9 ± 26.4</td>
<td>97.1 ± 25.3</td>
<td>90.9 ± 28.7</td>
<td>0.34</td>
</tr>
<tr>
<td>BMI</td>
<td>32.04 ± 8.65</td>
<td>31.47 ± 6.98</td>
<td>33.11 ± 11.30</td>
<td>0.90</td>
</tr>
<tr>
<td>Comorbidities - no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>41 (33.1%)</td>
<td>28 (68.3%)</td>
<td>13 (31.7%)</td>
<td>0.48</td>
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<tr>
<td>Heart Failure</td>
<td>12 (9.7%)</td>
<td>6 (50.0%)</td>
<td>6 (50.0%)</td>
<td>0.30</td>
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<tr>
<td>Diabetes</td>
<td>27 (21.8%)</td>
<td>21 (77.8%)</td>
<td>6 (22.2%)</td>
<td>0.08</td>
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<tr>
<td>COPD</td>
<td>9 (7.2%)</td>
<td>5 (55.6%)</td>
<td>4 (44.4%)</td>
<td>0.70</td>
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<tr>
<td>Chronic Kidney Disease *</td>
<td>35 (28.2%)</td>
<td>28 (80.0%)</td>
<td>7 (20.0%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Urine volume (ml)</td>
<td>55.2 ± 24.3</td>
<td>55.0 ± 17.6</td>
<td>55.7 ± 34.2</td>
<td>0.655</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.9 ± 1.8</td>
<td>2.2 ± 1.8</td>
<td>1.7 ± 2.0</td>
<td>0.019</td>
</tr>
<tr>
<td>Albuminuria (ACR &gt; 30)</td>
<td>33 (63.5%)</td>
<td>20 (60.6%)</td>
<td>13 (39.4%)</td>
<td>0.382</td>
</tr>
<tr>
<td>CRP &gt;3 mg/L [34 on record (65.4%)]</td>
<td>23 (67.6%)</td>
<td>15 (65.2%)</td>
<td>8 (34.8%)</td>
<td>0.458</td>
</tr>
<tr>
<td>Urinary Tract Infection- no. (%)</td>
<td>12 (23.1%)</td>
<td>8 (66.7%)</td>
<td>4 (33.3%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Mortality - no. (%)</td>
<td>12 (23.1%)</td>
<td>10 (83.3%)</td>
<td>2 (16.7%)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

* CKD presence determined by chart diagnosis during medical record abstraction. A breakdown of CKD stages follows: Stage 1 (n=1); Stage 2 (n=9), Stage 3a/b (n=11), Stage 4 (n=9), Stage 5 / ESRD (n= 5). Continuous variables are summarized as mean ± standard deviation. P-values were calculated with Kruskal-Wallis test (continuous variables) or Fisher’s exact test (categorical variables).
Table 2: Clinical characteristics of deceased COVID-19 patients.

<table>
<thead>
<tr>
<th></th>
<th>Alive</th>
<th>Deceased</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total - no. (%)</td>
<td>40 (76.9%)</td>
<td>12 (23.1%)</td>
<td></td>
</tr>
<tr>
<td>AKI - no. (%)</td>
<td></td>
<td></td>
<td>0.179</td>
</tr>
<tr>
<td>Yes</td>
<td>24 (60.0%)</td>
<td>10 (83.3%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>16 (40.0%)</td>
<td>2 (16.7%)</td>
<td></td>
</tr>
<tr>
<td>Cause of death - no. (%)</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Acute respiratory failure</td>
<td>--</td>
<td>7 (58.3%)</td>
<td></td>
</tr>
<tr>
<td>Multi system organ failure</td>
<td>--</td>
<td>1 (8.3%)</td>
<td></td>
</tr>
<tr>
<td>Arrythmia</td>
<td>--</td>
<td>1 (8.3%)</td>
<td></td>
</tr>
<tr>
<td>Metastatic cancer</td>
<td>--</td>
<td>1 (non-AKI) (8.3%)</td>
<td></td>
</tr>
<tr>
<td>Not on record</td>
<td>--</td>
<td>1 (AKI) + 1 (non-AKI) (16.7%)</td>
<td></td>
</tr>
<tr>
<td>Length of stay until death (days)</td>
<td>--</td>
<td>23 ± 16</td>
<td>NA</td>
</tr>
<tr>
<td>Duration of COVID-19 at sampling (days)</td>
<td>28 ± 19</td>
<td>20 ± 11</td>
<td>0.197</td>
</tr>
<tr>
<td>Required ventilation - no. (%)</td>
<td>23 (57.5%)</td>
<td>7 (58.3%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Developed sepsis - no. (%)</td>
<td>12 (30.0%)</td>
<td>2 (16.7%)</td>
<td>0.475</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>132 ± 21</td>
<td>124 ± 25</td>
<td>0.335</td>
</tr>
<tr>
<td>On blood pressure medication - no. (%)</td>
<td>31 (77.5%)</td>
<td>8 (66.7%)</td>
<td>0.466</td>
</tr>
</tbody>
</table>

Continuous variables are summarized as mean ± standard deviation. P-values were calculated with Kruskal-Wallis test (continuous variables) or Fisher’s exact test (categorical variables).
Figure Legend

Figure 1: Study design and inclusion criteria. Flowchart showing the exclusion and inclusion criteria used in the present study to select the study population of COVID-19 patients with or without acute kidney injury (AKI) during COVID-19-related hospital stay.

Figure 2: Presence of SARS-Cov-2 genetic material in urine sediments of COVID-19 patients and quantification of viral load in patients with or without AKI. A) Urine sediment viral load was higher in COVID-19 patients with AKI, while it was not significantly different from background in COVID-19 patients without AKI. B) Urine sediment viral load was significantly higher in COVID-19 patients with AKI during the first two weeks after the AKI diagnosis. C) A larger percentage of COVID-19 AKI patients had detectable viral loads in urine sediments compared with COVID-19 patients without AKI (P<0.05, Fisher’s exact test). (## P<0.05 vs. background; * P<0.05 vs. non-AKI, Kruskal-Wallis test). N: (COVID-19)= 51, (healthy controls)= 14. Each dot represents an individual datapoint, average viral copies/ng RNA is indicated (dotted line) and standard error. Dark-colored dots represent the same individuals in A) and B) with the highest viral loads.

Figure 3: Relationship of urine sediment viral load with mortality and albuminuria. A) Increased U-viral load among deceased patients (* P=0.03, Kruskal-Wallis test). B) Cumulative incidence of inpatient mortality at above and below a viral load of 5,141 copies/ng RNA. Sixty-day hazard ratio (HR): 11.50 (95% CI: 3.27-40.30), P<0.001. The probabilities of non-survival (at risk) for the duration of the observation period were: below 5,141 copies/ng RNA= 0.086, above 5,141 copies/ng RNA= 0.615. C) The average U-viral load was 1.5 times higher in COVID-19 patients with albuminuria at time of sampling, but not significant (P=0.50, Kruskal-Wallis test). D) Average U-viral load in COVID-19 patients at different stages of AKI (P=0.12, Kruskal-Wallis test). E) Average U-viral load in COVID-19 AKI patients who needed renal replacement therapy (RRT) or not (P=0.10, Kruskal-Wallis test). Each dot represents an individual datapoint, average viral copies/ng RNA is indicated (red line) and standard error (light blue). N= 48.
Figure 4: Expression of SARS-CoV-2 proteins Spike and Nucleocapsid (green), and ACE2 (red) in kidney biopsies. Blue label is DAPI nuclear staining. A) Proximal tubule (PT) in kidney cortex of COVID-19-positive patient no.1. ACE2 signal was apical, outlining the lumen of proximal tubules, and SARS-CoV-2 Spike was observed in an intracellular location (arrows). B) Glomerulus (G) in the same patient as (A). Insert shows higher magnification of area in outlined square, where SARS-CoV-2 Spike expression was detected in parietal cells. C) Spike expression in area of tubular necrosis in the kidney cortex of COVID-19-positive patient no. 2. Insert shows higher magnification of area in outlined square where Spike expression was detected in cortical tubules (nephron segment outlined by dotted line). D) SARS-CoV-2 Nucleocapsid expression in cortical tubule of COVID-19-positive patient no. 2. Arrowheads indicate cells of the tubular segment expressing nucleocapsid at different intensities. Insert shows higher magnification of area in outlined square. E) ACE2 expression and absence of Spike expression in proximal tubule (PT) of COVID-19-negative patient. F) Absent Spike expression in glomerulus (G) of COVID-19-negative patient. G) Absent Nucleocapsid expression in kidney cortex of a COVID-19-negative patient.

Figure 5: Expression of SARS-CoV-2 Spike (green) and ACE2 (red) in cells in urine sediment from COVID-19 patient. A) ACE2 and SARS-CoV-2 (Spike) co-expression in the same cell isolated from urine sediment of COVID-19-positive patient. B) ACE2 expression and absence of Spike in single cell from urine sediment of COVID-19-negative patient. Blue label is DAPI nuclear staining.
Figure 1

Enrollment

Participant enrollment in TCRC Biobank between 4/15/2020 and 06/08/2020 (N=652)

Excluded (N=560)
- Unable to produce urine at time of Collection
- Anuric Patients
- Processing limitations

Inclusion

Initial inclusion (N=92)
- Urine sedimentation conducted within 4 hours of collection
- Administrative diagnosis of AKI

Blinded Adjudication

Excluded from analysis
- SARS-CoV-2 PCR negative
- Clinically suspected (N=40)

SARS-CoV-2 PCR positive diagnosis (N=52)
- Administrative diagnosis of AKI (N=42)
- No administrative diagnosis of AKI (N=10)

Analysis

COVID-19 positive with AKI (N=34)

COVID-19 positive without presence of AKI (N=18)
Figure 2

A) Viral copies/ng RNA

B) Viral copies/ng RNA

C) Urine SARS-Cov-2 (+) | Urine SARS-Cov-2 (-)

Percentage distribution (%)
Figure 3

A) Viral copies/mg RNA

B) Cumulative Mortality Incidence

C) Albumin/Creatinin ratio (mg/g)

D) Viral copies/mg RNA

E) Viral copies/mg RNA

Legend:
- U-viral > 5140.7 counts/ng
- U-viral ≤ 5140.7 counts/ng

Groups:
- Alive
- Deceased
- Non-AKI
- AKI-Stage 1
- AKI-Stage 2
- AKI-Stage 3
- AKI-no RRT
- AKI-RRT
Figure 4
Figure 5

A  COVID-19 (+)

ACE2
Spike

10 µm

B  COVID-19 (-)

ACE2
Spike

10 µm
High SARS-CoV-2 Viral Load in Urine Sediment Correlates with Acute Kidney Injury and Poor COVID-19 Outcome

METHODS

Mar 15–Jun 8, 2020

52 COVID-19 patients

urine sediment

SARS-CoV-2 qRT-PCR

OUTCOME

Urine viral load

No AKI  AKI

alive  deceased

urine SARS-CoV2  

AKI  

Deceased

Conclusion

A higher SARS-CoV-2 viral load was measured in urine sediments from COVID-19 patients that developed AKI during hospitalization, and it predicted increased risk of mortality.

doi: 10.1681/ASN.2021010059

254x190mm (96 x 96 DPI)
Supplemental Material

Table of Contents

Supplemental Table 1. Clinical risk factors for patients with U-viral load higher than 5140 copies/ng RNA.

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Supplemental Figure S4. SARS-CoV-2 immunofluorescence in kidney biopsy.

Supplemental Figure S5. Expression of Nucleocapsid and ACE2 in cells shed in urine sediment from SARS-CoV-2 urine-positive patients.

Supplemental Figure S6. Kidney histopathology of COVID-19 patient with AKI.
**Supplemental Table 1.** Clinical risk factors for patients with U-viral load higher than 5140 copies/ng RNA.

<table>
<thead>
<tr>
<th></th>
<th>&lt;5140 copies/ng</th>
<th>&gt;5140 copies/ng</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total - no. (%)</strong></td>
<td>40 (76.9%)</td>
<td>12 (23.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Race - no. (%)</strong></td>
<td></td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>African American</td>
<td>32 (86.5%)</td>
<td>5 (13.5%)</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>6 (54.5%)</td>
<td>5 (45.5%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>0</td>
<td>2 (100%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1 (100%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (100%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Comorbidities - no. (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>31 (75.6%)</td>
<td>10 (24.4%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Heart Failure</td>
<td>9 (75.0%)</td>
<td>3 (25.0%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Diabetes</td>
<td>19 (70.4%)</td>
<td>8 (29.6%)</td>
<td>0.329</td>
</tr>
<tr>
<td>COPD</td>
<td>6 (66.7%)</td>
<td>3 (33.3%)</td>
<td>0.415</td>
</tr>
<tr>
<td>Chronic Kidney Disease</td>
<td>26 (74.3%)</td>
<td>9 (25.7%)</td>
<td>0.729</td>
</tr>
<tr>
<td><strong>Albuminuria (ACR &gt; 30)</strong></td>
<td>24 (72.7%)</td>
<td>9 (27.3%)</td>
<td>0.499</td>
</tr>
</tbody>
</table>
**Supplemental Figure S1:** A) Gel electrophoresis showing amplification product in RT-PCR for SARS-CoV-2 S gene. B) Gel electrophoresis showing amplification product in RT-PCR for SARS-CoV-2 N gene. A single band of the expected molecular weight was observed for both S and N genes. C) Representative standard curve (S) with known amounts of SARS-CoV-2 genome used for quantification of viral load in urine sediments. The lower limit of linearity was 10 viral copies. Ct values for all PCR reactions are indicated within the range of the standard curve. Healthy controls (symbol: +) were all below the limit of detection. Non-AKI (symbol: △) and AKI (symbol: ○) patients are indicated. Filled symbols are the patients considered positive after normalizing per ng RNA and they were all within the linear range of the standard curve.
### Supplemental Figure S2: Starting amount of RNA purified from urine sediments for qRT-PCR

<table>
<thead>
<tr>
<th></th>
<th>Total RNA isolated (ng)</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>450.7</td>
<td>116.3</td>
</tr>
<tr>
<td>COVID-19 -non AKI</td>
<td>1225.9</td>
<td>334.0</td>
</tr>
<tr>
<td>COVID-19 + AKI</td>
<td>1033.3</td>
<td>169.4</td>
</tr>
</tbody>
</table>

Total RNA isolated from urine sediment specimens from COVID-19 patients was significantly higher than from healthy controls (P=0.02, Kruskal-Wallis, n=14 healthy, n=48 COVID-19), most likely due to viral RNA enriched by the RNA purification kit. No difference was observed between AKI and non-AKI specimens (P>0.999, Kruskal-Wallis, n=17 non-AKI, n=32 AKI).
Supplemental Figure S3: U-viral load in relation with method of sampling, ventilation and parameters of kidney injury. A) U-viral load was not different between specimens with or without blood in urine (P=0.513, Kruskal-Wallis, n=48). B) U-viral load was not different between specimens obtained by spontaneous voiding or catheterization (P= 0.066, Kruskal-Wallis, n=48). C) U-viral load was not different between specimens obtained from patients on ventilators or not (P= 0.391, Kruskal-Wallis, n=48). D) No correlation was observed between U-viral load and serum creatinine levels (R² = 0.00005, Spearman rho = 0.241; n=48). E) U-viral load was not different between specimens obtained from patients with serum creatinine below or above 1 mg/dL (P = 0.0699, Kruskal-Wallis, n=48). F) No correlation was observed between U-viral load and C-reactive protein (CRP) (R² = 0.014, Spearman rho = 0.096; n=31). G) U-viral load was not different between specimens obtained from patients with CRP below or above 3 mg/L (P = 0.275, Kruskal-Wallis, n=31).
Supplemental Figure S4: Expression of SARS-CoV-2 Spike (green) and ACE2 (red) in kidney biopsy. Blue label is DAPI nuclear staining. **A)** Spike expression in collecting duct in COVID-19-positive patient no.1. Insert shows higher magnification of area outlined in square, where intracellular labeling of Spike was detected at a sub-apical location of collecting ducts (arrows) and interstitial cell (arrowhead). **B)** ACE2 and Spike expression in proximal tubule of same COVID-19-positive patient as in (A).
Supplemental Figure S5: Expression of Nucleocapsid and ACE2 in cells shed in urine sediment from SARS-CoV-2 urine-positive patients. Detection of SARS-CoV-2 nucleocapsid protein (Gentex, clone 6H3) and ACE2 in urine sediment cells (indicated by DAPI nuclear staining) from three additional patients with positive urine SARS-CoV-2 qRT-PCR.
Supplemental Figure S6: A) Acute tubular necrosis of kidney showing dilation of proximal tubular epithelium, sloughing, intraluminal fluid and interstitial plasmacytic inflammation and edema (H&E stain, medium power magnification view, formalin fixative). B) Collapsing glomerulopathy of kidney showing collapsed glomerular apparatus with cell necrosis immediately adjacent to dilated proximal tubules (H&E stain, medium power magnification view, formalin fixative). C) Electron Microscopic image showing extensive podocyte foot process effacement relative to the glomerular basement membrane in kidney glomerulus (glutaraldehyde fixative).