Acute kidney injury in COVID-19: are kidneys the target or just collateral damage? A comprehensive assessment of viral RNA and AKI rate in patients with COVID-19

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Purpose of review
To investigate the possible effects of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) on kidney function and assess the rate of viral ribonucleic acid (RNA) shedding/detection in urine.

Recent findings
Most of the research on the topic suggests that for the moment our ability to estimate whether SARS-CoV-2 is a direct causative agent in acute kidney injury (AKI) or whether it has a cytokine storm effect is limited. During our prospective assessment of 333 patients with COronaVIrus Disease 2019 (COVID-19) it was found that frequency of AKI of 9.6% (32 cases). Despite previous data suggestive of the ability to detect SARS-CoV-2 in urine, we were unable to identify any traces of messenger ribonucleic acid (mRNA) in our group. Both COVID-19 severity (odds ratio, OR = 23.09, confidence interval, CI 7.89–67.57, $P < 0.001$) and chronic kidney disease (CKD) history (OR = 7.17, CI 2.09–24.47, $P = 0.002$) were associated with the AKI rate.

Summary
AKI is a relatively frequent condition for patients with COVID-19 and is normally correlated with the severity of the disease and the patient's history of CKD. The available data fail to address whether SARS-CoV-2 mRNA is present in urine, whereas our prospective trial data suggest that mRNA is undetectable in urine irrespective of the severity of the disease.

Keywords
acute kidney injury, COVID-19, kidney

INTRODUCTION

Given the extent of the pandemic, COronaVIrus Disease 2019 (COVID-19) has become a primary concern for a great number of researchers around the globe. Following this trend, the urological community also started discussing the possible effects of COVID-19 on the urinary system. The effects of coronavirus Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) on the human body are determined by its structure. Viral spikes enable it to grip the receptors of angiotensin-converting enzyme 2 (ACE-2). ACE-2 receptors are present outside of the lungs making heart, bowel, gonad and kidneys potential targets [1]. A large number of studies suggest that the presence of ACE-2 receptors

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KEY POINTS

- The severe inflammatory process that occurs during COVID-19 infection affects almost all the organs of the human body including the kidney.

- The direct effect of COVID-19 on kidneys is still not properly understood.

- No SARS-CoV-2 RNA was identified in any of the urinary samples collected during the study.

- The question of whether direct kidney damage is caused by SARS-CoV-2 is up for debate.

in the kidneys would appear to be the main reason for acute kidney injury (AKI) in patients with SARS-CoV-2 [2]. These data followed by the reports suggesting that the viral ribonucleic acid (RNA) may be isolated from urine [3] has ignited debates regarding the rate SARS-CoV-2 secretion and virulence in urine [4▪]. The available data on the effect of SARS-CoV-2 on the kidney and urinary system are inconsistent suggesting an AKI rate in 0.1–29% of patients [5,6▪].

One possible explanation for this could be the presence of ACE-2 in the cell membrane and/or cytokine storm phenomenon which may lead to damage of the renal parenchyma in more advanced stages of the disease [2,5]. Today, determining whether SARS-CoV-2 is the cause of direct AKI is challenging due to the limitations in pathology assessment and molecular analysis. Most of the research on the topic suggests that for the moment our ability to estimate whether SARS-CoV-2 is a direct causative agent in AKI or it is cytokine storm effect and systemic response collaterally damage the kidneys is limited.

The goal of our study was to investigate whether SARS-CoV-2 affects the kidneys directly or indirectly through systemic inflammation. In order to assess this, we estimated the rate of kidney damage in COVID-19 patients and assessed the detectability/shedding of viral RNA in the urine of patients suffering from COVID-19 as a possible indicator of direct viral damage.

RECENT FINDINGS

The direct targets of SARS-CoV-2 are still very much up for debate. One possible explanation for this effect are the ACE2 and S-protein molecular pathways [7,8]. However, virus penetration remains the first stage of the process with the second crucial point being the innate immune response ('cytokine storm') [9]. Reports of enhanced expression of ACE-2 receptors were followed up by data showing an increased probability of AKI in COVID-19 patients. Gupta et al. showed that the AKI frequency among critically ill patients reached rates as high as 20.6% [10], varying in the general population between 5 and 7% [2,11]. Chan et al. confirmed an AKI rate in COVID-19 patients of 7.58% (95% CI 3.30–13.54%) [12]. The recent meta-analysis of Xu et al. showed that the frequency of AKI in COVID-19 patients lies somewhere around 10%, and that kidney damage is more frequent in older patients and patients suffering with a more acute form of the disease [13]. Putting all these factors together, these data led to the hypothesis that SARS-CoV-2 targets the kidney. This hypothesis was compounded by data regarding the presence of messenger ribonucleic acid (mRNA) and virus in the kidney tissue [14▪▪], electron microscopy data indicated COVID-19 could be associated with tissue damage [15▪], and evidence of SARS-CoV-2 mRNA was to be found in the patients' urine [4▪,16,17]. Moreover, one of the first papers on the topic even suggested that we may observe radiological changes in the kidneys. Zhen et al. mention that they were able to observe a decreased density of parenchyma which corresponded to swelling and edema [18].

Here at Sechenov, we have also performed an analysis to understand the extent of the effects of SARS-CoV-2 on the kidneys (clinicaltrials.gov ID NCT04386564). We conducted an analysis that included patients with pneumonia as identified by a chest computed tomography (CT) scan. This confirmed that [confirmed what? This sentence does not make sense] with nucleic acid detection of SARS-CoV-2 from throat swab samples using reverse transcription-polymerase chain reaction (RT-PCR). Only patients with recent disease onset (the onset was defined as the date when symptoms appear – less than a week) and who had undergone no antiviral therapy (COVID-19 specific treatment) were included. Patients who were receiving ongoing treatment with known renal toxicity were excluded. Patients were treated subsequently in accordance with local practices adhering strictly to the international guidelines available at the time of the study.

AKI according to acute kidney injury network (AKIN) criteria was used: an increase in serum creatinine (SCr) by $\geq 26 \mu$mol/L (0.3 mg/dL) within 48 h, or an increase in SCr to >1.5 times baseline within the previous 7 days, or urine volume <0.5 mL/kg/h for >6 h. The AKIN classification was chosen over RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) due to it is higher sensitivity shown in previous series [19]. Chronic kidney disease (CKD) was classified according to Kidney Disease: Improving Global Outcomes (KDIGO). Despite using these outcomes, we should

remember that our knowledge regarding COVID-19 is changing all the time. Various definitions of AKI have been used, but still no exact definition of AKI in COVID-19 has been proposed.

In order to assess whether the virus is able to infect the kidney, we performed on all the patients both a urine test and a throat swab for mRNA of SARS-CoV-2 at admission, and subsequently on a weekly basis and once again on discharge.

Both urine samples and throat-swabs were collected, and RNA was extracted with PREP-NA Extraction Kit (DNA-Technology Research & Production', LLC, Moscow, Russia). Real-time RT-PCR was used to detect the RNA of SARS-CoV-2 using CE-IVD SARS-CoV-2/SARS-CoV Multiplex real-time PCR Detection Kit ('DNA-Technology Research & Production', Moscow, Russia). Three target sites were amplified and tested simultaneously, namely, *N* (nucleocapsid phosphoprotein) and two sites in gene E (envelope protein) – the first specific for SARS-CoV -2, the second common to all SARS-CoV-like coronaviruses. The RNA reverse transcription stage and PCR amplification of cDNA stage were performed in one test tube. To increase the sensitivity and specificity of the amplification reaction, the use of a hotstart was provided. A multiplex real-time PCR detection kit includes the internal control which is intended to assess the quality of both the RNA extraction and

the PCR. All the tests were run in duplicates and a quantified positive control was adopted to ensure the results precision.

After 3 months of enrollment we were able to assess the data of 333 patients (June–August 2020). The patients were separated into groups according to the WHO COVID-19 classification of severity. Groups were compared using Analysis of Variation, t-test and Mann-Whitney U test. The mean age of patients was 55 years (SD was ± 14.3), with 178 males and 155 females. A total of 122 patients (36.6%) had congestive heart failure, 29 (8.7%) had diabetes mellitus type II, 27 had chronic kidney disease (8.1%) and one had undergone a radical prostatectomy 1 month prior to inclusion in the study (Table 1).

The overall AKI rate according to the KDIGO criteria was 9.6% (32 cases). The mortality rate was 4.5% (15 cases). Despite a tendency for deteriorating kidney function in patients showing severe symptoms and patients with ARDS, the only difference was lower estimated glomerular filtration rate (eGFR) in ARDS group at discharge ($P = 0.02$). COVID-19 severity was associated with an increased rate of AKI ($P < 0.001$). eGFR was better in the patients with moderate COVID-19 ($P = 0.020$) at discharge compared the those will severe COVID-19 or ARDS (moderate - 98.4 mL/min/1.73 m^2 vs.

Table 1. Analysis of kidney function and overall status in 333 patients with moderate-to-sever COVID-19 infection

	Overall	COVID-19 severity according to WHO			P
		Moderate	Severe	ARDS	
N (%)	333 (100)	213 (63.9)	46 (13.8)	74 (22.2)	
Age, years mean ± SD	55.1 ± 14.3	57.9 ± 13.9	55.0 ± 18.0	56.6 ± 12.8	0.301
AKI, (% of group)	32 (9.6)	7 (3.2)	7 (15.2)	18 (24.3)	P < 0.001*
SCr (Serum creatinine) mcmol/L, mean ± SD					
1 day (admission)	94.9 ± 18.7	93.5 ± 11.5	99.1 ± 16.8	101.5 ± 18.7	0.826
peak level	89.9 ± 15.1	94.8 ± 17.7	90.7 ± 15.7	87.6 ± 13.1	0.725
at discharge	86.8 ± 13.9	85.8 ± 8.3	90.8 ± 12.9	84.8 ± 13.9	0.548
eGFR (estimated glomerular filtration rate) mL/min/1.73 m^2, mean ± SD					
1 day (admission)	70.2 ± 17.8	75.6 ± 23.8	71.2 ± 14.7	63.2 ± 12.3	0.343
peak level	75.8 ± 17.0	80.2 ± 21.2	78.9 ± 13.2	68.9 ± 13.6	0.296
at discharge	78.7 ± 16.4	98.4 ± 19.5	85.7 ± 10.4	77.6 ± 12.7	0.020*
BUN (blood urea nitrogen), mmol/L, mean ± SD					
1 day (admission)	5.6 ± 2.2	5.7 ± 3.8	5.4 ± 1.8	5.9 ± 1.6	0.563
peak level	5.9 ± 2.5	5.5 ± 2.4	5.6 ± 1.5	5.7 ± 1.3	0.842
at discharge	5.5 ± 1.7	5.2 ± 1.3	5.0 ± 1.4	5.3 ± 1.2	0.518
C-reactive protein, mg/L, median (IQR)					
1 day (admission)	44.0 (IQR 19.0–82.8)	35.2 (IQR 19.7–53.2)	47.6 (IQR 26.9–61.2)	45.9 (IQR 34.8–45.9)	0.512
peak level	35.7 (IQR 13.0–82.4)	25.9 (IQR 8.4–41.8)	35.2 (IQR 19.7–53.2)	74.0 (IQR 34.5–79.0)	0.087
at discharge	1.9 (IQR 1.0–12.1)	0.9 (IQR 0.2–3.0)	1.9 (IQR 1.0–5.0)	8.9 (IQR 3.1–25.4)	0.061

*Statistically significant difference.

Table 2. Uni- and multivariable analysis assessing the probability of acute kidney injury (AKI) in 333 patients with moderate-to-severe COVID-19 infection

	Univariable			Multivariable		
	P	**OR**	**CI 95%**	**P**	**OR**	**CI 95%**
COVID-19 severity (WHO)	0.001*	48.73	19.20 123.7	0.001*	23.09	7.89 67.57
Previous CKD	0.001*	32.98	12.91 84.24	0.002*	7.17	2.09 24.47
Diabetes mellitus	0.606	0.67	0.15 2.98	0.272	0.33	0.04 2.38

*Statistically significant.

severe -85.7 mL/min/1.73 m^2 and ARDS - 77.6 mL/min/1.73 m^2) (Table 1). In most of the AKI patients (28/32), hematuria/proteinuria was detected at least a day prior to AKI onset.

In order to assess the possible predictors and covariates of AKI, we performed both uni- and multivariate analyses. After univariate analyses, we performed a p-value selection, and then added the number of contributing criteria to the model. After a multivariate analysis, the following criteria were suggested as contributing factors — CKD history (OR = 7.17, CI 2.09–24.47, $P = 0.002$) and COVID-19 severity (OR = 23.09, CI 7.89–67.57, $P < 0.001$) were the only predictors for AKI. There was no association between diabetes mellitus and/or heart failure with AKI (Table 2). Furthermore, there was no association between other assessed factors (i.e., the patient's age, gender, past medical history, days from symptoms onset and etc.) and AKI onset.

Moreover, using the Spearman and Pearson correlation where appropriate, we found that CKD had a strong positive correlation with COVID-19 disease severity ($r = 0.53$, $P < 0.001$) and AKI ($r = 0.54$, $P < 0.001$). Age and history of congestive heart failure had mild correlations with severe COVID-19 associated pneumonia [$r = 0.14$ ($P = 0.02$) and $r = 0.13$

($P = 0.002$), respectively]. There was no correlation between congenital heart failure (CHF) and AKI [$r = 0.112$ ($P = 0.054$), whereas age had correlated with disease severity and $r = 0.12$ ($P = 0.03$), respectively] (Table 3).

As the last part of the trial patients' urine was tested for SARS-CoV-2 RNA at admission, then on a weekly basis and again at discharge. Despite a rigorous diagnostic, we were unable to identify SARS-CoV-2 mRNA in urine, even in patients with significant viral load according to RT-PCR taken from a throat swab. As already known, previous CHF and patient age were correlated with disease severity. However, with the exception of CKD, none of the assessed factors were predictive of AKI. One possible explanation for this is the limited cohort size. As for the predictors, as expected, hematuria and/or proteinuria were present in most of the patients with AKI (28/32) and were predictive of kidney function impairment. Yet, even in those patients no SARS-CoV-2 RNA was found in urine. CT which was performed through the course of the study was unable to identify any significant changes in kidney parenchyma with only a minimal slowing down of contrast uptake in a few patients.

PRO AND CONTRA

After a year of research, it remains up for discussion whether SARS-CoV-2 targets the kidneys initially or not. Khan *et al.* in their paper mentioned four main points which led them to believe that SARS-CoV-2 may infect the kidneys – among them viral RNA in kidneys and urine; presence of viral protein immunocytochemistry; data on live viral presence in renal tissues and identification of coronavirus-like structures in kidney tissue [20■]. Although the authors believe that support for this evidence is currently only anecdotal, it should nonetheless not be ignored.

It should also be noted that the AKI frequency in COVID-19 patients is of great concern. We and several other groups have observed an increased rate of AKI in patients with CKD history and in those

Table 3. Pearson and Spearman correlations of the associations of clinical factors with the likelihood of acute kidney injury (AKI) and COVID-19 severity according to the WHO COVID-19 classification in 333 patients with moderate-to-severe COVID-19 infection

Parameter	AKI	COVID-19 severity (WHO)
Age	0.12 (0.03)*	0.14 (0.02)*
BMI	0.91 (0.15)	0.91 (0.15)
Previous CKD	0.538 ($P < 0.001$)*	0.54 ($P < 0.001$)*
Diabetes mellitus	−0.03 (0.60)	0.33 (0.55)
Chronic heart failure	0.112 (0.52)	0.13 (0.02)*

Presented as Spearman or Pearson coefficients where appropriate (*p*-value).
*Significant correlation.

suffering from other severe diseases [13]. Yet, none of this indicates a direct effect of COVID-19 on the kidneys. At present, the COVID-19 infection is showing a wide range of clinical signs, but none of them can be purely associated with upfront renal function impairment. It should be mentioned that none of the kidney-related symptoms were observed at disease onset and only occurred during the progression of COVID-19, both in our study and in the available literature on topic.

As for urine viral shedding, Chan *et al.* in their systematic review performed a pooled analysis which showed a minimal detection rate of mRNA in the urine of 5.74% (95% CI 2.88–9.44%) [12]. The publication of this data raised a number of questions concerning the possibility of SARS-CoV-2 passing the renal membrane. In relation to this, most authors suggested that further assessment of the viral load in urine is necessary with a focus on its virulence as it could affect safety and urinary hygiene protocols [4▪,14▪▪]. In line with previous research, our trial was unable to identify SARS-CoV-2 mRNA in urine.

We sincerely believe that more substantial findings are needed to confirm the direct effects of COVID-19 on kidney function and exclude the possibility of indirect damage due to SARS-CoV-2-induced hypoxemia as suggested by Wang *et al.* [17]. Moreover, as Meizlish *et al.* [21] have shown there are a number of circulating factors (VEGF-A (Vascular endothelial growth factor), PDGF-AA (Platelet Derived Growth Factor), and PDGF-AB/BB) with angiogenic effect which were particularly pronounced in non-intensive care unit symptomatic patients. Ackermann *et al.* mentioned that COVID-19 pneumonia was associated with perivascular T-cell infiltration and severe endothelial injury with thrombosis. Patients with COVID-19 had nine times the normal amount of microthrombi ($P < 0.001$) and 2.7 times the normal amount of angiogenesis ($P < 0.001$). These findings may support the hypothesis that hypoxemic injury underlies the AKI [22]. Therefore, after a year of research there is still a great deal of uncertainty regarding the exact pathogenesis of AKI in COVID-19 patients. We sincerely believe that subsequent research may shed light on the exact effects of COVID-19 and on viral shedding (not only with RT-PCR but also detecting viral proteins). A further issue which needs to be addressed in more detail is whether the SARS-CoV-2 affects the kidneys of those showing minimal signs of disease.

CONCLUSION

AKI is a relatively frequent condition for patients with COVID-19 and is normally correlated with the severity of the disease and the patient's history of CKD. The available data fail to address whether SARS-CoV-2 mRNA is present in urine, whereas our prospective trial data suggests that mRNA is undetectable in urine irrespective of the severity of the disease.

Acknowledgements

Financial support and sponsorship

Conflicts of interest

There are no conflicts of interest.

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