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Acute Kidney Injury and Urinary and Histopathological Disorders in Kidney Transplant Patients with SARS-CoV-2 Infection

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ABSTRACT

Background. Acute kidney injury (AKI) is a manifestation of SARS-CoV-2 infection. The evidence in kidney transplant (KT) is limited, as there are scarce data about the histologic features in graft biopsies of these patients.

Material and Methods. A retrospective cohort study of KTs with SARS-CoV-2 infection from August 28, 2020, to April 23, 2021. We collected the incidence of AKI and the presence of urinary and histopathological disorders. Both groups were compared (AKI vs no AKI). Immunohistochemical and reverse transcription-polymerase chain reaction studies were performed on the anatomopathological samples.

Results. In our study, 72 KTs had SARS-CoV-2 infection and, among them, 27 patients (35.1%) developed AKI related to increased severity and a worse evolution of the infection, defined by a greater presence of pneumonia ($P < .001$), hospitalization ($P < .001$), admission to the intensive care unit ($P < .001$), the need for ventilation support ($P < .001$), and continuous renal replacement therapy ($P < .001$). In the multivariable analysis, pneumonia behaved as an independent predictor for AKI development ($P = .046$). No differences were observed between proteinuria a month before and after infection ($P = .224$). In addition, 5 patients showed microhematuria and 2 patients presented transient glycosuria without hyperglycemia. Of the 5 kidney biopsies performed, 1 biopsy (20%) showed positive reverse transcription polymerase chain reaction for SARS-CoV-2.

Conclusions. AKI is a frequent and potentially serious complication in KT patients. Occasionally it could be accompanied by abnormalities in the urinary sediment. Of 5 biopsied patients, 1 patient had positive reverse transcription polymerase chain reaction in renal tissue, which suggests the systemic spread of the virus and the tropism for the renal graft.

KIDNEY involvement in SARS-CoV-2 infection is common and its clinical presentation may range from mild proteinuria to acute kidney injury (AKI). Although the first works from China reported a low incidence of AKI in COVID-19 patients (3%-9%) [1], the current evidence shows that AKI rates are higher, reaching up to 43% in the rest of the series [2]. Furthermore, the incidence of AKI is markedly greater in critical patients because severe COVID-19 is likely to cause failure of multiple organs [3]. In this sense, up to 79% of critical patients may develop AKI, and some cases need renal replacement therapy (RRT). Thus, AKI is also considered a marker of severity and a negative prognostic factor for survival [2,4].

In addition, some studies have described changes in urinary sediment in patients with SARS-CoV-2 infection, such as proteinuria, hematuria, and glycosuria, suggesting the presence of a renal reservoir for the virus [5−7]. Notwithstanding, kidney biopsies are not routinely performed in these patients, so there is no specific histologic data.

The information on AKI incidence and urinary abnormalities in kidney transplant (KT) recipients with SARS-CoV-2

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0041-1345/20
https://doi.org/10.1016/j.transproceed.2022.04.008

infection is limited. However, these patients present several factors that can cause kidney impairment in addition to a higher risk of critical COVID-19 due to chronic immunosuppression and coexisting comorbidities. Therefore, specific studies in this population are necessary. Herein, we describe the incidence of AKI and the urinary and histopathological abnormalities as well as the risk factors for AKI in a cohort of KT recipients with SARS-CoV-2 infection.

MATERIAL AND METHODS

We performed a retrospective cohort study of all KT patients in our center with SARS-CoV-2 infection from August 28, 2020, (first case) to April 23, 2021. In all cases, the diagnosis was made by reverse transcription-polymerase chain reaction (RT-PCR) test in a nasopharyngeal swab.

We defined AKI according to the Kidney Disease: Improving Global Outcomes classification [8]. Clinical and analytical variables were collected, and patients with AKI were compared with those who did not develop AKI. We estimated glomerular filtration using the Modification of Diet in Renal Disease equation [9].

Patients were followed until recovery, defined by negative RT-PCR or Inmunoglobulin G (Ig G) positive serology or until death.

We also analyzed changes in the urine analysis during the infection (proteinuria, hematuria, or glycosuria). Proteinuria was determined by the albumin/creatinine ratio (ACR) in a first-morning urine sample. We compared the ACR the month before infection and 1 month later.

KT biopsies were performed if there was impaired renal function/urinary abnormalities such as previously described. In our center, samples of the upper pole of the kidney graft are taken by expert nephrologists using the 16-gauge biopsy under ultrasound-guide. All kidney biopsies were processed by standard techniques for light microscopy and immunofluorescence.

Renal tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. The processed sections were 5 μm each. Immunohistochemical studies were performed to detect SARS-CoV-2 with SARS-CoV spike antibody, Rabbit Pab (IgG) and SARS-CoV Nucleoprotein antibody, Mouse Mab (IgG), from Sino Biological laboratory. These antibodies are reactive on routine paraffin-embedded histologic sections, using the avidin-biotin complex technique and heat-induced epitope retrieval (Optiview DAB IHC v6, Window). Immunohistochemical staining was performed with a 1:100 dilution for the nucleoprotein and 1:300 for the spike in an automated system (Ventana Benchmark ultra, Tucson, AZ). Heat-induced epitope retrieval was performed (4 minutes at 95°C for nucleoprotein and 8 minutes for spike), and the sample was subsequently incubated for 32 minutes for nucleoprotein and spike at room temperature.

The histologic samples were analyzed using the Applied Biosystems TaqMan 2019-nCoV Assay v1 kit (Cat. No. A47532) that contains a set of TaqMan RT-PCR assays for the qualitative detection and characterization of SARS-CoV-2 RNA. The kit identifies different viral genomic regions (ORFab1, protein S, and protein N) and the human RNase P gene. Three target sequences are used to increase the reliability of the technique and reduce the risk of false positives. The kit includes a positive control, TaqMan 2019-nCoV Control Kit v1 (Cat. No. A47533) for the 3 regions.

Continuous variables are presented as mean and standard deviation or median and interquartile range as appropriate, and categorical variables as absolute value and percentage. The normality of the samples was analyzed by the Kolmogorov Smirnov test. All categorical variables were compared using Fisher exact test or X^2 test. Continuous variables were compared using the Student *t* test or U Mann-Whitney for independent groups. In the case of paired groups, the Student *t* test for paired data was used or the Wilcoxon signed-rank test according to the normality of the sample. A multivariable logistic regression analysis was performed for AKI risk factors after SARS-CoV-2 infection. We used SPSS V.26.0 software (IBM, Armonk, NY, United States) for statistical analysis.

RESULTS

In the study period, 77 KTs had SARS-CoV-2 infection in our center. The mean age was 54.2 years, and 57.1% were men. Six KT recipients were in the first year post-KT, and the median follow-up after infection was 20 days. Twenty-seven patients (35.1%) developed AKI. According to the severity of the AKI, they were classified as follows: AKI-I, n = 13 (48.1%); AKI-II, n = 6 (22.2%); and AKI-III, n = 8 (29.6%).

We compared baseline characteristics between groups (AKI vs no AKI) (Table 1). Patients with AKI mostly had diabetes (P = .044) and had lymphopenia more frequently (P < .001). The presence of AKI was related to higher severity of the infection, defined by a greater presence of pneumonia (74.1% vs 10%, P < .001), hospitalization (88.9% vs 20%, P < .001), admission to the intensive care unit (ICU) (40.7% vs 2%, P < .001), and the need for ventilatory support (37% vs 2%, P < .001). We found no differences in age, sex, hypertension, immunosuppressive treatment, baseline renal function, or time post-KT.

The incidence of AKI in inpatients was 70.5% (n = 24), and, in critical patients (n = 12), it reached 91.7% (n = 11) and was more severe, defined by a higher proportion of patients with AKI-III (54.5% vs 12.5%, P = .033) and RRT (45.4% vs 0%, P = .006).

The mortality rate in patients with AKI was higher (51.9% vs 0%, P < .001), and those with AKI III had a mortality rate higher (75%) than in patients with AKI I (30.8%) and AKI II (66.7%). All patients with AKI who required RRT (n = 5) died. Patients cured of COVID-19 recovered baseline renal function.

In the multivariable analysis, pneumonia was an independent predictor for AKI in KT patients with SARS-CoV-2 infection (P = .046) (Table 2).

For urinary abnormalities, we did not observe any changes in ACR between the value 1 month before infection and 1 month later (31.75 [14.97, 100.32] mg/g vs 31.30 [10.32, 181.90] mg/g; P = .224). Five patients showed microhematuria, and 2 patients showed transient glycosuria without hyperglycemia.

Renal biopsy was performed in 5 patients, and 4 of them were recent KT (Table 3). The median time post-KT to biopsy was 12 days (range, 6-515). In 3 patients, the biopsy was performed because of impaired renal function, showing chronic toxicity due to anticalcineurin agents in 1 of them. In contrast, the rest showed no significant alterations. The fourth patient was a highly-sensitized KT patient (98% PRA), whose renal biopsy showed an acute antibody-mediated rejection. The last case was a recent KT with non-normalization of renal function. Renal biopsy presented acute tubular necrosis (ATN), and the

Table 1. Clinical Characteristics of KT Patients With SARS-CoV-2 Infection (AKI vs No AKI)

Clinical Characteristics	All (n = 77)	AKI (n = 27)	No AKI (n = 50)	*P* value
Age (y), mean (SD)	54.2 (12.4)	56.6 (11.3)	53.1 (12.9)	.251
Sex (male), n (%)	44 (57.1)	16 (59.3)	28 (56.0)	.838
HBP, n (%)	73 (94.8)	26 (96.3)	47 (94.0)	.488
DM, n (%)	31 (40.3)	15 (55.6)	16 (32.0)	.044
Prednisone, n (%)	76 (98.7)	27 (100.0)	49 (98.0)	1.000
Cyclosporine, n (%)	4 (5.2)	0 (0.0)	4 (8.0)	.291
Tacrolimus, n (%)	71 (92.2)	26 (96.3)	45 (90.0)	.417
mTOR inhibitors, n (%)	8 (10.4)	4 (14.8)	4 (8.0)	.440
Mycophenolate, n (%)	62 (80.5)	22 (81.5)	40 (80.0)	.876
Asymptomatic, n (%)	18 (23.4)	4 (14.8)	14 (28.0)	.192
Time from KT (mo), median [IQR]	93 (41.166)	84 (24.167)	97 (46.167)	.447
Baseline GFR (mL/min), mean (SD)	46.6 (18.4)	42.7 (14.6)	49.5 (20.7)	.231
Fever, n (%)	35 (45.5)	18 (66.7)	17 (34.0)	.006
Dyspnea, n (%)	25 (32.5)	19 (70.4)	6 (12.0)	< .001
Gastrointestinal symptoms, n (%)	13 (16.9)	6 (22.2)	7 (14.0)	.358
Pneumonia, n (%)	25 (32.5)	20 (74.1)	5 (10.0)	< .001
Hospitalization, n (%)	34 (44.2)	24 (88.9)	10 (20.0)	< .001
ICU admission, n (%)	12 (15.6)	11 (40.7)	1 (2.0)	< .001
Lymphopenia, n (%)	26 (33.8)	17 (63.0)	9 (18.0)	< .001
CRRT, n (%)	5 (6.5)	5 (18.5)	0 (0.0)	< .001
Ventilator support, n (%)	11 (14.3)	10 (37.0)	1 (2.0)	< .001
Dead, n (%)	14 (18.2)	14 (51.9)	0 (0.0)	< .001

AKI, acute kidney injury; CRRT, continuous renal replacement therapy; DM, diabetes mellitus; GFR, glomerular filtration rate; HBP, high blood pressure; ICU, intensive care unit; IQR, interquartile range; KT, kidney transplant; SD, standard deviation.

RT-PCR test in the anatomopathological sample was positive for SARS-CoV-2.

SARS-CoV-2 IgG antibodies against spike and nucleoprotein in anatomopathological samples were negative in all renal biopsies performed.

DISCUSSION

We present one the first studies on AKI and urinary abnormalities after SARS-CoV-2 infection in a cohort of KT patients. Furthermore, we have confirmed the presence of viral genes in the histopathological samples.

In our study, the AKI rate in inpatients with COVID-19 was 70.5%, reaching up to 91.7% in critical patients. This rate is markedly higher than in the general population, where the incidence ranges from 27% to 43% and 19% to 78%, respectively [2]. Mortality in KT patients with AKI is also higher than in the series currently published in the non-KT population [10]. These data highlight the greater severity and the poor outcomes of COVID-19 in KT patients.

Table 2. Multivariable Analysis Using Binary Logistic Regression for Risk Factors for Acute Kidney Injury

Risk Factors	Exp (ß)	OR (95% CI)		*P* value
Age (y)	0.987	0.927	1.051	.682
DM	1.389	0.295	6.533	.677
ICU admission	8.272	0.766	89.332	.082
Pneumonia	14.675	2.170	99.229	.006
Fever	0.620	0.118	3.260	.572
Lymphopenia	1.669	0.346	8.049	.524

DM, diabetes mellitus; ICU, intensive care unit; OR, odds ratio.

Patients with AKI had a higher hospitalization rate, increased ICU admission, and ventilator support. In addition, they needed continuous RRT and developed pneumonia more frequently, which is consistent with other series reports in both KT patients [4] and the general population. Due to this greater severity, AKI determines a bad prognosis of the infection, especially in hospitalized patients [11,12].

AKI has been associated with higher mortality in patients with COVID 19, especially in more severe cases of AKI. An U. S. study reported a mortality rate of 35% in patients with AKI; of those who died, 91% had AKI-III. The fatality rate was 55% in those needing RRT [8]. Another study showed an in-hospital mortality rate of 33.7% in those with COVID-19-associated AKI compared with 13.4% in those with AKI without COVID-19. Patients with AKI-III and COVID-19 had a 2.6-fold higher mortality rate than those with stage AKI-III who did not have COVID-19 [13]. In our study, half of patients with AKI died and those with AKI-III had a fatality rate higher than in patients with AKI-I or II. In our series, all patients with AKI who needed RRT died. These results show that renal impairment in KT patients with COVID is related to worse outcomes than in general population, reflecting the greater vulnerability of KT recipients to the infection.

Hirsh et al reported that age and diabetes mellitus were risk factors for developing AKI in patients with SARS-CoV-2 infection [11]. In our study, we have also documented a greater rate of diabetes in KT recipients with AKI. Although these patients were older, age was not statistically significant, probably due to a higher incidence of COVID−19 in our

Table 3. Kidney Transplant Biopsy Findings from Positive COVID-19 Cases

												KT biopsy findings		
	Age (y)	Sex	Donor Type	Time from KT (d)	Time from COVID-19 diagnosis to KT biopsy (d)	sCr (mg/dL)	ACR (mg/g)	Hematuria	Glycosuria	DSA	KT biopsy indication	Light microscopy	RT-PCR	IQ
Case 1	34	Male	DD	44	33	1.78	34.5	-	-	-	AKI	No findings	-	-
Case 2	61	Male	DD	107	58	1.62	177.1	-	-	-	AKI	No findings	-	-
Case 3	64	Male	DD	547	2	2.63	511.4	-	-	+	AKI	Chronic injury due to CNI	-	-
Case 4	48	Male	DD	15	9	3.95	2695.5	+	+	+	No recovery of renal function	ABMR	-	-
Case 5	51	Male	DD	23	15	1.99	141.8	-	+	-	No recovery of renal function	ATN	+	-

ABMR, antibody-mediated rejection; ACR, albumin-to-creatinine ratio; AKI, acute kidney injury; ATN, acute tubular necrosis; CNI, anticalcineurin inhibitors; DSA, donor specific antibodies; DD, deceased donor; IQ, immunohistochemistry; KT, kidney transplant; RT-PCR, reverse transcription-polymerase chain reaction; sCr, serum creatinine.

cohort of elderly KT patients. Additionally, patients with AKI presented more frequently lymphopenia ($P < .001$), as reported in other studies of KT patients with COVID-19 [2,12].

In our work, pneumonia was an independent predictor for the development of AKI in KT patients with COVID-19. The fundamental pathophysiology of pneumonia in critically ill patients is severe acute respiratory distress syndrome and the development of a multi-organ failure, which have been identified as independent risk factors for AKI [3,14]. Pei et al described the severity of pneumonia as a negative independent predictive factor of kidney complications in non-KT patients [15].

For alterations in the urinary sediment, hematuria and proteinuria after COVID-19 have been described in several studies [4,16]. Notwithstanding, in our series, we found no differences between albuminuria in the month before and after infection. We did not include albuminuria values at the time of infection due to the absence of data, especially in critical patients, where their clinical situation did not allow us to quantify it. Consequently, we cannot determine if there was transient proteinuria. Hematuria was present in 5 patients and glycosuria without hyperglycemia was present in 2 patients. These latest discoveries could be related to glomerular and tubular damage of the virus, as other authors have already elucidated [5−7].

In this study, we have also described the histologic findings in KT patients with COVID-19. Renal histopathology was analyzed by Santoriello et al in 42 autopsies of deceased patients with COVID-19 without finding specific alterations [17]. The most prevalent finding in these biopsies was ATN (62%) [17]. Kudose et al evaluated 17 biopsy samples of native and allograft kidneys from patients with COVID-19 [16]. The most frequent findings in these biopsies were ATN (29%) and collapsing glomerulopathy (29%) [16]. In our biopsied KT patients, 1 of them, a recent KT without recovery of renal function, had ATN as the main finding. Notwithstanding, he also presented a positive RT-PCR for SARS-CoV-2 in renal tissue. These findings suggest that COVID-19 can spread through the bloodstream and infect other organs such as the kidney graft. Immunohistochemistry failed to detect the virus in all kidney tissue samples analyzed, as in other studies [18,19]. Our results could show the greater sensitivity of RT-PCR with respect to immunohistochemistry for the detection of SARS-CoV-2 in histologic samples of renal tissue. We cannot clearly show the influence that time after infection can have on the negativity of both tests.

Study limitations

Our study presents several limitations. This is a retrospective, single-center study with the limitations that inherently may exist in data collection. Therefore, we want to emphasize the exploratory nature of this study, and we hope the findings will serve as a base for future studies. Finally, we do not have histologic samples from patients with a fatal evolution of SARS-CoV-2 infection that probably reflects better the histologic damage caused by this virus. Additionally, we do not have proteinuria values at the time of infection for the reason described.

CONCLUSIONS

AKI is a frequent and potentially serious complication in KT patients that causes high hospitalization rates and ICU admission, mechanical ventilation, continuous renal replacement therapy, and pneumonia. Occasionally, it could be accompanied by abnormalities in the urinary sediment. One of the 5 biopsied patients had positive RT-PCR in renal tissue, which suggests the systemic spread of the virus through the bloodstream and the tropism for the renal graft.

REFERENCES

[1] Wang L, Li X, Chen H, et al. Coronavirus disease 19 infection does not result in acute kidney injury: an analysis of 116 hospitalized patients from Wuhan, China. Am J Nephrol 2020;51:343–8.

[2] Kellum JA, van Till JWO, Mulligan G. Targeting acute kidney injury in COVID-19. Nephrol Dial Transplant 2020;35:1652–62.

[3] Gabarre P, Dumas G, Dupont T, Darmon M, Azoulay E, Zafrani L. Acute kidney injury in critically ill patients with COVID-19. Intensive Care Med 2020;46:1339–48.

[4] Cravedi P, Mothi SS, Azzi Y, et al. COVID-19 and kidney transplantation: results from the TANGO International Transplant Consortium. Am J Transplant 2020;20:3140–8.

[5] Akalin E, Azzi Y, Bartash R, et al. COVID-19 and kidney transplantation. N Engl J Med 2020;382:2475–7.

[6] Calomeni E, Satoskar A, Ayoub I, Brodsky S, Rovin BH, Nadasdy T. Multivesicular bodies mimicking SARS-CoV-2 in patients without COVID-19. Kidney Int 2020;98:233–4.

[7] Miller SE, Brealey JK. Visualization of putative coronavirus in kidney. Kidney Int 2020;98:231–2.

[8] Kellum JA, Lameire N, Aspelin P, Barsoum RS, Burdmann EA, Goldstein SL, et al. Kidney disease: improving global outcomes (KDIGO) acute kidney injury work group. KDIGO clinical practice guideline for acute kidney injury. Kidney Int Suppl 2012;2(1): 1–138.

[9] Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. Ann Intern Med 1999;130: 461–70.

[10] Chan L, Chaudhary K, Saha A, et al. AKI in hospitalized patients with COVID-19. J Am Soc Nephrol 202132:1;51-60.

[11] Hirsch JS, Ng JH, Ross DW, et al. Acute kidney injury in patients hospitalized with COVID-19. Kidney Int 2020;98:209–18.

[12] Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054–62.

[13] Fisher M, Neugarten J, Bellin E, et al. AKI in hospitalized patients with and without COVID-19: a comparison study. J Am Soc Nephrol 2020;31:2145–57.

[14] Darmon M, Clec'h C, Adrie C, et al. Acute respiratory distress syndrome and risk of AKI among critically ill patients. Clin J Am Soc Nephrol 2014;9:1347–53.

[15] Pei G, Zhang Z, Peng J, et al. Renal involvement and early prognosis in patients with COVID-19 pneumonia. J Am Soc Nephrol 2020;31:1157–65.

[16] Santoriello D, Khairallah P, Bomback AS, et al. Postmortem kidney pathology findings in patients with COVID-19. J Am Soc Nephrol 2020;31:2158–67.

[17] Kudose S, Batal I, Santoriello D, et al. Kidney biopsy findings in patients with COVID-19. J Am Soc Nephrol 2020;31:1959–68.

[18] Xia P, Wen Y, Duan Y, et al. Clinicopathological features and outcomes of acute kidney injury in critically ill COVID-19 with prolonged disease course: a retrospective cohort. J Am Soc Nephrol 2020;31:2205–21.

[19] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395:507–13.