Viral Clearance and Serological Response to SARS-CoV-2 in Kidney Transplant Recipients

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ABSTRACT

Objectives. Knowledge about the impact of coronavirus disease 2019 (COVID-19) on kidney transplant recipients (KTRs) concerning viral shedding and humoral immune response against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is limited. The aim of this study is to analyze viral dynamics and the antibody response to SARS-CoV-2 in KTRs with COVID-19 and study their association with clinical data.

Materials and methods. Consecutive KTRs diagnosed with COVID-19 at our center were evaluated for clinical presentation and outcome; duration of viral shedding and viral burden by reverse transcription-polymerase chain reaction assay cycle threshold; and magnitude of seroconversion to SARS-CoV-2.

Results. Six KTRs identified with COVID-19 were hospitalized. Presenting symptoms were similar to those in the general population. Four patients had severe disease and, of these, 2 required mechanical ventilation, 4 had acute kidney injury, and 3 had secondary bacterial infections. Immunosuppression was reduced in all patients. Five patients were treated with hydroxychloroquine. No patient required dialysis or died. Patients with severe disease had a longer duration of viral shedding, which lasted more than 40 days, and had IgG antibodies against SARS-CoV-2, which were detected from 3 weeks to as long as 10 weeks after symptom onset. In patients with less severe disease no IgG antibodies where detected between 9 and 14 weeks after symptom onset.

Conclusions. In our series, KTRs with severe COVID-19 had prolonged viral shedding and a stronger humoral immune response to SARS-CoV-2. These preliminary data need to be confirmed with further studies and over a longer period of time.

CORONAVIRUS disease 2019 (COVID-19), caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China, in December 2019 and has rapidly spread throughout the world, resulting in a global pandemic. The treatment of kidney transplant recipients (KTRs) with SARS-CoV-2 remains largely unknown. Immunosuppressive therapy increases the risk of infectious complications of RNA respiratory viral infections, including bacterial and fungal superinfections [1]. Moreover, KTRs frequently have comorbidities associated with severe COVID-19, such as hypertension, diabetes mellitus, and cardiovascular disease [2]. Despite the limited information on the impact of COVID-19 in KTRs, it is hypothesized that immunosuppression could attenuate the hyperinflammatory state [3],

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predispose KTRs to a more intense and prolonged shedding of the virus [4], and reduce humoral immune responses against SARS-CoV-2 [5].

In Portugal, through June 9, there were a total of 35,306 diagnosed cases of COVID-9 and 1492 deaths [6]. During the same period, 6 out of 1058 consecutive KTRs were diagnosed with COVID-19 at our center. The aim of this study is to analyze viral dynamics and the antibody response to SARS-CoV-2 in KTRs with COVID-19 and study their association with clinical data.

MATERIAL AND METHODS

Patients

All KTRs who were diagnosed with COVID-19 at our center through June 9, 2020, were included. Confirmed diagnosis of COVID-19 was based on a positive real-time reverse transcription-polymerase chain reaction (RT-PCR) assay performed on oropharyngeal and nasopharyngeal swabs. Patient characteristics were retrieved from the electronic medical records. Disease severity was scored from mild to severe for all patients [2].

According to our hospital protocol, all of the following criteria had to be met for hospital discharge: 1. apyrexia for at least 2 consecutive days, 2. significant clinical improvement without respiratory insufficiency nor need of supplemental oxygen, 3. no radiological worsening, and 4. availability of home isolation facilities. Patients still in hospital at the end of follow-up were considered to be clinically recovered if they met the first 3 criteria for hospital discharge. Follow-up period was defined as the time between symptom onset and June 9, 2020. The study was approved by the ethical review board of our hospital. All individuals gave written or oral informed consent. The work was performed in accordance with the Declaration of Helsinki. All data are anonymized.

Viral RNA Detection

Sequential nasopharyngeal and oropharyngeal swab samples were collected and sent to the laboratory in the same virus preservation solution container. RT-PCR assay for SARS-CoV-2 was performed using LightMix Modular SARS and Wuhan CoV E-gene kit (TIB Molbiol, Berlin, Germany) with LightCycler Multiplex RNA Virus Master (Roche, Basel, Switzerland), according to manufacturers' instructions, to determine the presence of virus through the identification of the envelope gene. Cycle threshold (Ct) values were obtained from positive samples stored at −20°C. RT-PCR Ct values were used as indicators of the copy number of SARS-CoV-2 RNA, with lower Ct values representing higher viral RNA loads. Samples were considered negative if the Ct values exceeded 39 cycles. Duration of viral shedding was defined as the time between the first and the last positive SARS-CoV-2 test. Patients who tested negative for SARS-CoV-2 in 2 consecutive pharyngeal swabs separated by at least 1 day were considered to be clear of infection.

Serologic Tests

SARS-CoV-2 antibodies were measured with 2 different assays targeting IgG antibodies against the S1 subunit of the spike protein (enzyme-linked immunosorbent assay, Analyzer I, Euroimmun, Luebeck, Germany) and IgG against the nucleocapsid (N) protein (chemiluminescent microparticle immunoassay, Abbott Architect Immunoassay Analyzer, Chicago, Ill, United States). Testing was performed on serum samples following manufacturers' instructions. Thresholds for positivity were supplied by the assay manufacturers. Cross-sectional serologic testing was done from May to June 2020. Retrieval of serum remnant from blood samples taken for routine biochemical testing was performed in 2 patients. Sensitivities of 89.3% and 96% and specificities of 98.3% and 99.3% were reported for Euroimmun IgG [7] and Abbott IgG [8], respectively. Seronegative patients were negative for both IgGs.

Statistical Analysis

Categorical variables were expressed as frequencies and continuous variables were expressed as medians with ranges or interquartile ranges. All statistical analyses were performed using SPSS version 20.0 for Macintosh (IBM, NY, United States).

RESULTS

Baseline Characteristics

A total of 6 KTRs were identified at our center (Table 1). The first case was detected on March 12, 2020, and the last on April 26, 2020. Patients' median age was 56 years (range, 36-72) and half were female. The median time from transplant to COVID-19 diagnosis was 161 months (range, 8-235). Only patient 2 had been transplanted for less than 1 year and induction therapy was antithymocyte globulin-Fresenius. None of the patients had previous rejection episodes. Regarding maintenance immunosuppression therapy, all patients were on low-dose prednisolone, a calcineurin inhibitor (CNI), and mycophenolate mofetil. Comorbidities included hypertension (4/6), cardiovascular disease (3/6), and obesity (3/6), and 1 patient had post-transplant diabetes. In the previous 6 months, median creatinine was 1.20 mg/dL (range, 0.8-1.45).

Clinical Presentation and Outcome

Clinical features are summarized in Table 1. Presenting symptoms were fever (5/6), followed by cough (3/6), diarrhea (2/6), myalgias (2/6), and dyspnea (1/6). Median time from symptom onset to diagnosis was 4 days (range, 0-8). At presentation all patients had high C-reactive protein, half had lymphocytopenia ($<1 \times 10^9$/L), and 2 had thrombocytopenia. Other findings included mild to moderate lactate dehydrogenase and aspartate aminotransferase elevation. Five patients presented with chest x-ray or computed tomography scan abnormalities. Patient 6 did not have an initial chest x-ray but a computed tomography scan done later showed bilateral consolidations and ground glass opacities.

Two patients had moderate disease with viral pneumonia but no need for supplemental oxygen, and the clinical course was uncomplicated. Four patients had severe disease with variable clinical course. Patients 4 and 6 developed severe disease 4 and 10 days after symptom onset, respectively, requiring conventional oxygen therapy. Patient 2 was transferred to the intensive care unit (ICU) on day 6 of hospital stay and required mechanical ventilation for 19 days. Patient 5 had a rapid decline in clinical condition in the first 24 hours and was admitted to the ICU needing mechanical ventilation for 29 days. Two patients had acute

Table 1. Patient Characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age at diagnosis (years)	72	36	45	57	55	56
Sex	Male	Female	Male	Male	Female	Female
Time from transplant to infection (months)	166	8	75	155	235	212
Immunosuppression	PND, CsA, MMF	PND, TAC, MMF	PND, TAC, MMF	PND, TAC, MMF	PND, CsA, MMF	PND, CsA, MMF
Comorbid conditions	HTN; CAD	Posttransplantation diabetes; obesity class II	HTN	HTN, HF	Obesity class II	HTN; HF; obesity class I
Symptom onset to diagnosis (days)	8	3	6	2	4	0
Presenting symptoms	Diarrhea, myalgias	Fever	Fever, cough, diarrhea, myalgias	Fever, cough, dyspnea	Fever, cough	Fever
Baseline investigations						
WBC ($\times 10^9$/L)	7.32	2.18	10.52	4.03	5.47	5.47
Lymphocytes ($\times 10^9$/L)	5.53	0.81	7.82	0.58	1.50	0.92
Platelets ($\times 10^9$/L)	236	80	162	107	265	192
C-reactive protein (mg/L)	152	83	161	79.8	87.2	58.5
LDH (IU/L)	ND	393	ND	309	306	232
AST (IU/L)	25	59	152	68	60	16
Chest radiology						
Ground glass opacities	No	Yes	No	Yes	Yes	Yes
Consolidations	Yes	Yes	Yes	Yes	Yes	Yes
Bilateral involvement	No	Yes	No	Yes	Yes	Yes
Clinical classification	Moderate	Severe	Moderate	Severe	Severe	Severe
ICU admission	No	Yes	No	No	Yes	No
AKI	No	Stage 2	No	Stage 1	Stage 1	Stage 1
Secondary infections	No	Yes	No	No	Yes	Yes
Hospital stay (days)	20	38	4	10	>56*	>50*
Duration of viral shedding (days)	1	52	1	>66†	ND	>44†
Follow-up (days)	97	83	80	68	60	44

Abbreviations: AKI, acute kidney injury; AST, aspartate aminotransferase; CAD, coronary artery disease; CsA, cyclosporine; HF, heart failure; HTN, hypertension; ICU, intensive care unit; LDH, lactate dehydrogenase; MMF, mycophenolate mofetil; ND, not determined; PND, prednisolone; TAC, tacrolimus; WBC, white blood cell count.

*Still in hospital at the end of follow-up.

†Still positive at the end of follow-up.

kidney injury (AKI) on admission and 2 developed AKI during the course of the disease. None required renal replacement therapy and all patients' serum creatinine levels returned to their usual values. The clinical course of patients 2, 5, and 6 was complicated by bacterial coinfections and patient 5 had cytomegalovirus colitis.

All patients stopped mycophenolate mofetil, and CNI was reduced or suspended in 1 patient (patient 5). Prednisolone dose was increased to 20 mg in patients 2 and 5. All patients except patient 6 were treated with hydroxychloroquine after the exclusion of a prolonged QT interval. Median follow-up for the entire cohort was 74 days (range, 44-97) and no patient was lost. At the end of follow-up, 4 of the 6 patients had recovered and had been discharged from the hospital. Patients 5 and 6 were still hospitalized, both clinically recovered; patient 5 was hospitalized because of critical illness myopathy and patient 6 because of persistent viral shedding with no home isolation facilities. No patient died of the infection.

Duration of Viral Shedding and Viral Load Assessed by Cycle Threshold Value

A total of 40 pharyngeal samples were collected, with a median of 6 samples from each patient (range, 3-13). RT-PCR Ct values were available in the first swab in 4 patients (patients 2, 3, 4, and 6) and in 46% of the subsequent positive swabs in these patients, from the 23rd day after symptom onset (ASO) onwards. In patient 5 duration of viral shedding was not determined because the second swab done 33 days after the first was negative. Duration of viral shedding was inferior to 9 days in both patients with moderate disease and was superior to 40 days in patients with severe disease (patients 2, 4, and 6). Viral shedding persisted despite clinical recovery in these patients. Patients 4 and 6 still had positive RT-PCR swabs at the end of follow-up (Table 1). Time between symptom onset and first RT-PCR Ct value ranged from 0 to 6 days. The median RT-PCR Ct value of the initial swab was 21.86 (range,

Table 2. SARS-CoV-2 RT-PCR and IgG Serologic Tests Results

		Weeks ASO													
Patient	Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	RT-PCR		POS*,† (Ct ND) NEG/NEG												
	IgG											NEG			NEG
2	RT-PCR	POS (Ct 19.65)	POS (Ct ND)		POS (Ct ND)	POS (Ct ND)	POS/POS† (Ct ND)	POS (Ct ND)	POS (Ct 28.87)	NEG	NEG				
	IgG			POS (S1: 10.7) (N: ND)		POS (S1: 4.2) (N: 6.83)	POS (S1: 4) (N: 7.1)				POS (S1: 1.3) (N: 3.35)				
3	RT-PCR	POS (Ct 24.75)	NEG		NEG										
	IgG									NEG					
4	RT-PCR	POS (Ct 24.0)	POS (Ct ND)		POS (Ct ND)	POS (Ct 35.45)	POS/NEG†,‡ (Ct 30.84)	POS† (Ct 33.22) (Ct 32.24)	POS (Ct ND)	POS† (Ct ND) (Ct 34.21) (Ct 34.08)	POS (Ct 34.6)				
	IgG								POS (S1: 7.2) (N: 7.5)						
5	RT-PCR	POS (Ct ND)					NEG/NEG†								
	IgG					POS (S1: 3.3) (N: 6.9)			POS (S1: 6.1) (N: 4.85)						
6	RT-PCR	POS (Ct 16.4)	POS (Ct ND)		POS† (Ct 28.4) (Ct 30.5)	POS† (Ct ND) (Ct 36.56)	POS (Ct 29.18)	POS (Ct 34.3)							
	IgG			POS (S1: NEG) (N: 6.5)	POS (S1: 3.6) (N: 6.81)	POS (S1: 2.8) (N: 7.35)	POS (S1: 2.7) (N: ND)								

Abbreviations: ASO, after symptom onset; Ct, cycle threshold values of reverse transcription-polymerase chain reaction targeting envelope gene; N, IgG index against the nucleocapsid (N) protein; ND, not determined; NEG, negative; POS, positive; RT-PCR, reverse transcription-polymerase chain reaction; S1, IgG ratio against the S1 subunit of the spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

*Patient 1 had a positive and 2 subsequent negative RT-PCR results during the second week ASO.

†In the week indicated in the table, the patient had more than 1 RT-PCR result.

‡In the week indicated in the table, the patient had 1 positive and 1 negative RT-PCR result.

16.45-24.75) and was increased in subsequent evaluations, with median Ct values of 33.22 (interquartile range = 30.56-34.3; Table 2).

Serologic Testing

The first serum sample for serologic testing was obtained at a median of 44 days (range, 15-76) ASO. A total of 14 serum samples were obtained (1 to 4 per patient). Serologic test results are shown in Table 2.

Seroconversion was observed in the 4 patients with severe disease, and IgG antibodies were observed from 3 to 10 weeks ASO. In patients 2 and 6 in whom serologic tests where done earlier, at 3 weeks ASO, seroconversion preceded clinical improvement. In patients with 2 or more follow-up samples, antibody kinetics varied. In patient 2, IgG levels against S1 and N decreased over time, and 10 weeks ASO the IgG against S1 was near the limit of positivity. In patient 6, IgG levels remained stable until 6 weeks ASO with negative or low reactivity of IgG against S1 in all samples. In patient 5 antibody titers either increased or decreased 8 weeks ASO, depending on the assay used. After seroconversion detection, viral shedding persisted for more than 15 days in patients 2, 4, and 6. Patients with moderate disease (patients 1 and 3) were clinically recovered and viral cleared at the time of sample collection. They tested negative for both IgG assays between the weeks 9 and 14 ASO.

DISCUSSION

In this case series, we describe clinical characteristics and outcomes of KTRs with COVID-19 and report correlations with viral shedding and humoral immune response to SARS-CoV-2.

Presenting symptoms and biochemical changes were similar to those described for the general population [9]. The majority of our patients presented suggestive findings of viral pneumonia on admission chest radiology. The most frequent comorbidities were hypertension, cardiovascular disease, and obesity, and only 1 patient was older than 60. The clinical course of COVID-19 in KTRs has varied but appears to be more severe, with greater rates of ICU admission and death [10]. Two-thirds of our patients had severe disease, and one-third required ICU admission and mechanical ventilation. Other major complications were observed, including AKI and secondary infections, as reported in other studies [10,11]. There were no clinical rejection episodes or deaths during follow-up. Most of our patients were treated with hydroxychloroquine, before clinical trials failed to show its efficacy [12]. Adjustment of immunosuppression in KTRs with COVID-19 remains largely unknown. On one hand, immunosuppression may be associated with poor virologic control, leading to more severe disease and prolonged viral shedding. On the other, it may attenuate the inflammatory response associated with severe disease [3]. Our local protocol included reduction of immunosuppression according to severity of disease and immunologic risk. Antimetabolites were suspended, CNI was reduced or suspended, and low-dose prednisolone was continued or increased to 20 mg per day in case of CNI suspension or higher immunologic risk.

Immunosuppression impairs host defense mechanisms, particularly T-cell-mediated immunity and the formation of T-cell-dependent antigen-specific B cells with antibody production [13]. Therefore, KTRs with compromised adaptive T-cell response are at increased risk of complications from viral respiratory infections and have a decreased ability to develop seroconversion to infectious diseases [5]. Prolonged viral shedding and higher viral loads, as occurs in other respiratory viral infections [14], are anticipated in immunosuppressed KTRs. In most individuals with symptomatic COVID-19, viral RNA in upper respiratory samples, as measured by PCR assay Ct values, peaks early within the first week of symptom onset and then starts to decline [15]. In patients with severe disease, viral shedding tends to persist for a longer period of time [16]. In our cohort of KTRs, lower Ct values (inversely related to viral RNA loads) were observed in the first swab, increasing to high values over time in those with sequential Ct values. Moreover, half of our patients, all with severe disease, had prolonged viral shedding that lasted for more than 40 days and persisted after clinical recovery, as observed in previous studies [17,18]. However, a positive PCR result reflects only the detection of viral RNA and does not indicate the presence of infectious virus. A significant relationship has been found between RNA viral load and time from symptom onset with viral culture positivity, with no infectious virus isolation from respiratory samples more than 8 days ASO [17,19]. Studies on immunocompromised patients are scant. One study in patients with severe and critical disease, which included 30 immunocompromised patients, suggested viral shedding of infectious virus for longer periods of time, up to 20 days [20]. Successful clearance of the virus requires adaptive immunity, and the role of T-cell-mediated immunity in the clearance of SARS-CoV-2 infection needs to be addressed. Except for the patient less than 1 year after transplant receiving antithymocyte globulin induction, there were no differences in immunosuppression between patients according to disease severity. Although we cannot draw any conclusion from our observation, the effect of the type and intensity of immunosuppression on cellular and humoral immune response to SARS-CoV-2 needs to be specifically studied.

Data from nonimmunosuppressed patients shows that following COVID-19, most patients develop detectable antibodies within days to weeks of symptom onset, with the rate of seroconversion increasing after the second week [17,21]. In our series, two-thirds of KTRs, all with severe disease, had detectable IgG antibodies against the S1 and N proteins between 3 and 10 weeks ASO. Persistence of viral RNA could still be detected in pharyngeal swabs after seroconversion, which was also observed in other studies [21]. In the 2 KTRs with serum samples from the third week onwards, seroconversion preceded clinical improvement. Nonetheless, the relationship between antibody response and clinical course is uncertain [17]. There are currently few

studies showing the performance of the serologic tests in immunocompromised patients, and data on the duration of antibodies after infection is not yet known. Though 1 report found a rapid decrease in antibodies within 2 to 3 months, particularly among patients with mild disease [22], 1 recent population-based study showed persistence of antibodies for 4 months after diagnosis [23]. In our study, 1 of our patients with severe disease, with the longest follow-up, had decreasing antibody levels at 10 weeks ASO, with the anti-S1 IgG antibody approaching the limit of positivity. In contrast to patients with severe disease, seroconversion was not observed in the 2 patients with moderate disease. Thus, either they did not developed a humoral immune response to SARS-CoV-2 or had undetectable levels of antibody because of less severe infection [22] and immunosuppression. Serologic studies in KTRs are scarce. In a study by Benotmane et al [18], most of the 40 KTRs with severe or nonsevere COVID-19 developed antibodies to SARS-CoV-2 with a stable titer until day 59 ASO, whereas in a study by Azzi et al [10] 20% of the 69 RT-PCR positive patients were negative for SARS-CoV-2 IgG antibody when screened at a median of 44 days after diagnosis. These discrepancies may be partly explained by differences in the sensitivity and specificity of the assays used, different sample sizes, and timing of testing. Our results are in in accordance with the study of Azzi et al [10], and although limited to a small number of KTRs, we used 2 assays targeting 2 different antigens in order to avoid false-negative results over a long period of follow-up. However, to clarify the relationship between humoral immune response and immunity to SARS-CoV-2, studies assessing neutralizing antibodies and T-cell-mediated immunity are necessary.

Our study has several limitations mainly due to the small number of patients included, but during the study period only 0.6% of KTRs followed at our center developed COVID-19. Factors that could affect seroconversion such as advanced age, comorbidities, intensity and type of immunosuppressive therapy, and lower graft function could not be evaluated. The absence of sequential serologic data to determine the time of seroconversion ASO and Ct values could not be determined in several swab samples.

CONCLUSIONS

This single-center case series observational study describes the viral dynamics of SARS-CoV-2 infection and serologic data in KTRs over an extended period of observation. Patients with severe COVID-19 had longer duration of viral shedding and detectable IgG antibodies to SARS-CoV-2 compared to patients with moderate forms of the disease. Our results are relevant for studies screening for past SARS-CoV-2 infections in KTRs, where severity of infection and timing of testing seems to be important. These preliminary data need to be confirmed in more comprehensive studies.

DATA AVAILABILITY

Data will be made available on request.

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