



Influence of immunosuppression on seroconversion against SARS-CoV-2 in two kidney transplant recipients

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Abstract

Solid organ transplant recipients are at risk for infectious complications due to chronic immunosuppression. The outbreak of coronavirus disease 2019 (COVID-19) in the United States has raised growing concerns for the transplant patient population. We seek to add to the current limited literature on COVID-19 in transplant recipients by describing the clinical course of two kidney transplant recipients with SARS-CoV-2 infection monitored by both RT-PCR and serology. Through careful adjustment of their immunosuppression regimen, both patients had excellent recovery with intact graft function and development of anti-SARS-CoV-2 antibodies.

KEYWORDS

antibodies, COVID-19, immunosuppression, RT-PCR, SARS-CoV-2, seroconversion

1 | INTRODUCTION

The novel coronavirus (SARS-CoV-2), which first led to an outbreak of acute severe respiratory disease (COVID-19) in Wuhan, China, has since spread across the globe. In the United States, the first case was identified on January 22, 2020, and has since increased to 2 545 250 confirmed cases, leading to 126 369 deaths as of June 29, 2020.¹ Solid organ transplant recipients may be at greater risk for severe complications due to immunosuppression and a high prevalence of comorbidities. While more data on COVID-19 in solid organ transplant recipients have been made available recently,^{2,3} the optimal management remains unclear especially in light of the disease's high mortality in transplant recipients.² Here, we describe the clinical course of SARS-CoV-2 infection in two kidney transplant recipients, both of whom recovered and seroconverted against SARS-CoV-2.

2 | CASE REPORT

2.1 | Patient 1

A middle-aged woman, who underwent deceased donor kidney transplant 2 months prior, presented for post-transplant clinic

follow-up with fatigue, loss of appetite, and temperature of 37.3°C for 1 week. Laboratory testing was notable for new-onset leukopenia to 2.1 K/ μ L (absolute lymphocyte count 0.13 K/ μ L). She had no respiratory symptoms and no gastrointestinal symptoms.

Her past medical history included end-stage renal disease from chronic pyelonephritis, almost 10 years of hemodialysis, sleeve gastrectomy, and type 2 diabetes.

She was highly allosensitized and received a flow cross-match-negative deceased donor kidney transplant with a low-level preformed donor-specific antibody. Immunosuppression consisted of anti-thymocyte globulin induction (5 mg/kg) and maintenance therapy of tacrolimus, mycophenolate (MMF), and prednisone. Her post-transplant course was complicated by 3 weeks of delayed graft function. She received cytomegalovirus (CMV) and pneumocystis jiroveci pneumonia prophylaxis with valganciclovir and trimethoprim-sulfamethoxazole, respectively.

In clinic, she underwent SARS-CoV-2 RT-PCR testing by nasopharyngeal swab (developed by Stanford Clinical Virology Laboratory^{4,5}) and went home with instructions to self-isolate pending results. Serological testing was not performed at the time since it was not yet available to our institution. The following morning SARS-CoV-2 RT-PCR resulted positive. She remained minimally symptomatic with fatigue and low-grade fever. She was instructed to stop MMF.

The next day, she reported new-onset cough, rhinorrhea, and dyspnea. At presentation to emergency department, she was hypoxic on minimal exertion, with an O₂ saturation of 85% on room air. Chest x-ray revealed diffuse bilateral patchy opacification. Her laboratory testing during hospitalization is summarized in Table 1. She was admitted with diagnosis of SARS-CoV-2 pneumonia.

She was maintained on 1-3 L of oxygen via nasal cannula with O₂ saturations of 91%-94%. Tacrolimus was continued but dose adjusted to a lower target level of 4-7 ng/mL, and prednisone was maintained at 5 mg daily. She did not receive antibiotics or antivirals. On day 3 of hospitalization (diagnosis day 4, symptom onset day 11), she had worsening fever (38.6°C) and increasing dyspnea. CT chest showed extensive bronchovascular "crazy paving" with associated regions of consolidation and regions of lobular sparing (Figure 1). In light of her clinical deterioration, hydroxychloroquine was initiated. By hospital day 7, she no longer required supplemental oxygen, and on day 11 (diagnosis day 12, symptom onset day 19), she was well enough to be discharged home. On day of discharge, IgM and IgG antibodies to the SARS-CoV-2 spike receptor-binding domain tested positive while repeat (nasopharyngeal) SARS-CoV-2 RT-PCR remained positive. She was discharged on tacrolimus and prednisone with MMF held. Repeat SARS-CoV-2 RT-PCR obtained on diagnosis day 26 (symptom onset day 33) was negative, and MMF was reinitiated.

2.2 | Patient 2

An elderly woman with end-stage renal disease presumed due to diabetic nephropathy who was 6 years status post deceased donor kidney transplant presented to an outside hospital emergency room with a week-long history of dry cough and fevers up to 38.8°C.

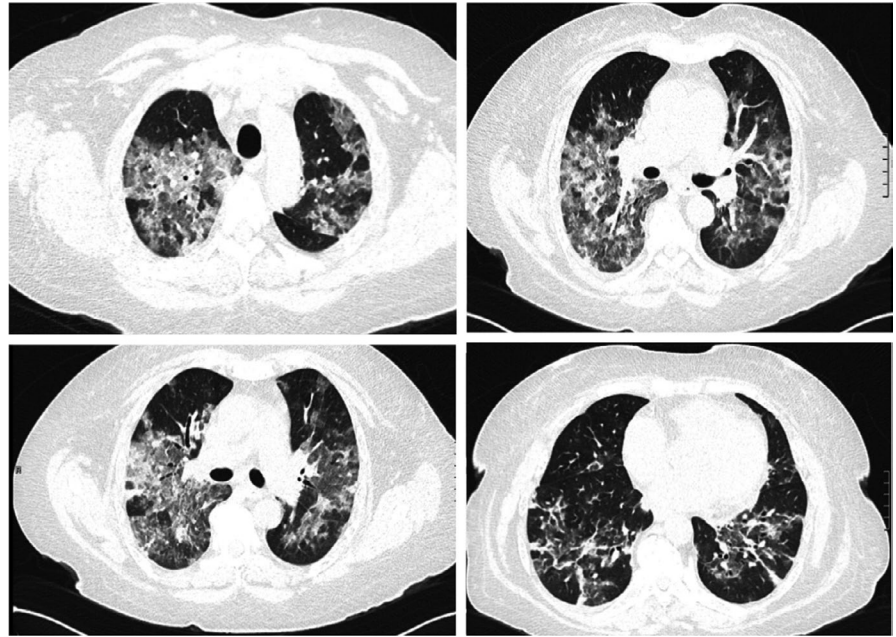
Her past medical history included type 2 diabetes, hypertension, and obesity. She was maintained on tacrolimus, MMF, and prednisone for immunosuppression with good kidney allograft function. Other medications include losartan 50 mg daily.

In the emergency department, she was hypoxic and required supplemental oxygen. Chest x-ray revealed bilateral interstitial infiltrates. SARS-CoV-2 RT-PCR returned positive (diagnosis day 0, symptom onset day 7). A serological test was not performed at the time. She was treated with hydroxychloroquine, ceftriaxone, and azithromycin based on hospital protocol. By day 7 (symptom onset day 14), she had improved clinically and was discharged home. She continued her home immunosuppression regimen throughout hospitalization, although following consultation with us, MMF was held on day 8. Repeat SARS-CoV-2 RT-PCR on day 23 (symptom onset day 30) was negative. MMF was reinitiated. IgM and IgG antibodies to the SARS-CoV-2 spike receptor-binding domain were performed on day 29 (symptom onset day 36), and both resulted positive.

TABLE 1 Laboratory parameters of patient 1

Serum Variable	Reference Range	Baseline	Diagnosis Day 0	Diagnosis Day 2	Diagnosis Day 4	Diagnosis Day 12
White Blood Cells (K/ μ L)	4.0-11.0	5.7	2.1	3.1	4.0	5.7
Neutrophils Abs (K/ μ L)	1.70-6.70	4.26	1.43	2.52	3.32	4.23
Lymphocytes Abs (K/ μ L)	1.00-3.00	0.47	0.13	0.08	0.11	0.41
Creatinine (mg/dL)	0.51-0.95	1.80	2.27	2.27	2.34	1.73
C-Reactive Protein (ml/dL)	<0.5			5.5		1.9
Ferritin (ng/mL)	13-150			3502	3342	2800
LDH (U/L)	135-214		291	290	309	272
D-dimer (μ g/mL)	<0.50			1.81	1.91	1.05
Lactate (mmol/L)	<2.0			1.2		
Procalcitonin (ng/mL)	<=0.50			0.18		
AST (U/L)	10-35	23	37	49	45	30
ALT (U/L)	10-35	14	15	18	19	18
Peripheral T + B Lymphocytes						
CD3 (%)	55-83					33
CD20 (%)	7-21					47
CD19 (%)	6-19					48
CD3+/CD4+ (%)	28-57					14
CD3+/CD8+ (%)	10-39					17
CD3 (/ μ L)	700-2100					114
CD20 Abs (/ μ L)	120-630					162
CD19 Abs (/ μ L)	100-500					165
CD3+/CD4+ (/ μ L)	300-1400					48
CD3+/CD8+ (/ μ L)	200-900					58

FIGURE 1 High-resolution computed tomography images on day 4 from diagnosis



3 | DISCUSSION

We describe two kidney transplant recipients with different clinical presentations and duration of immunosuppression, both achieved excellent clinical outcomes with supportive care and adjustment in immunosuppression.

Patient 1 had absence of respiratory or GI symptoms at time of positive COVID-19 diagnosis. However, she had severe COVID-19 disease per World Health Organization⁶ with profound lymphopenia, elevated D-dimer, ferritin, and CRP, all of which are associated with high risk for clinical deterioration. Seminari et al⁷ similarly reported an atypical presentation in a kidney transplant recipient (with only malaise, fever, and vomiting). Therefore, it may be prudent to have a lower clinical threshold for testing in solid organ transplant recipients to avoid missed diagnosis.

Patient 1 presented a difficult challenge as she was highly allosensitized and under 3-months post-transplant. Our goal was to permit the development of a host immune response against SARS-CoV-2 while, at the same time, continuing to provide adequate prophylaxis against graft rejection. We stopped MMF, continued home-dose prednisone and maintained tacrolimus with a reduced trough level goal. MMF is frequently the first medication dose reduced or held in response to viral infections in transplant recipients. MMF inhibits the enzyme inosine monophosphate dehydrogenase and prevents the proliferation of T and B lymphocytes.⁸ Specifically, the proliferation of natural killer cells and activation of viral-specific cytotoxic T lymphocytes are suppressed by MMF, which have been shown to negatively impact recovery from CMV infection.⁹ Early discontinuation of MMF may have allowed for the observed expansion in peripheral B lymphocyte population with CD19 and CD20 expression (Table 1). Continuing tacrolimus, on the other hand, may have been protective via its anti-inflammatory effect through decreased synthesis of IL-2, which is necessary for lymphocyte activation.¹⁰

For COVID-19-positive solid organ transplant recipients, the concern lies not only in the successful clearance of the virus, but also the development of an immunologic response. Serological responses in transplant recipients to infections and vaccines are frequently poor when compared with immunocompetent patients.^{11,12} MMF can be an especially potent inhibitor of the humoral immune system. The maintenance immunosuppression regimen of tacrolimus/MMF results in a greater suppression of the post-transplant humoral alloimmune response than cyclosporine/azathioprine.¹³ MMF may additionally inhibit desirable post-transplant immune responses such as seroconversion to vaccines. In a study of 94 kidney transplant recipients, the rate of seroconversion to the H1N1 influenza vaccine was lowest in patients treated with MMF.¹⁴ Multiple other studies in the kidney transplant population support the negative association between MMF and seroconversion following different vaccines.¹⁵⁻¹⁷ Furthermore, when it does occur the magnitude of antibody response is decreased and peak antibody response is delayed in transplant recipients on MMF-containing regimens compared to non-immunosuppressed controls.¹⁸ Based on these findings and our usual management of transplant recipients with severe viral infection, we have implemented routine temporary cessation of MMF in patients who test positive for SARS-CoV-2 by RT-PCR.

Zhong et al¹⁹ described SARS-CoV-2 disease in 2 solid organ transplant recipients and concluded that viral shedding was prolonged and antibody response was delayed when compared to non-immunocompromised counterparts. Xia et al²⁰ recently also described a positive SARS-CoV-2 RT-PCR renal transplant recipient who had failed to seroconvert completely. However, in our first patient, the detection of IgM and IgG antibodies to SARS-CoV-2 was on diagnosis day 12 (symptom onset day 19), indicating that an immune response can be mounted rapidly under immunosuppression, with response time comparable to the observed average time of immunocompetent patients (10-13 days^{21,22}). While detection of IgM has

high false-positive rate due to increased cross-reactivity between coronaviruses, thus making its diagnostic utility somewhat unclear,²³ the detection of IgG antibody is less likely to be false positive due to its higher antigen affinity. Our in-house IgG assay has a sensitivity of 100% and specificity of 97% when performed >21 days post symptom onset, comparable to two other commercially available assays Abbott and EUROIMMUN which has specificity of 99.9% and 94.8%, and sensitivity of 93.8% and 85.4%, respectively, at greater than 14 days post symptom onset.²⁴ The rapidity of antibody formation in our first patient may be attributed to the early discontinuation of MMF. The finding of positive IgM and IgG antibodies in our second patient with a negative SARS-CoV-2 RT-PCR on diagnosis day 23 illustrated that viral shedding may not be significantly prolonged in a solid organ transplant recipient. This finding is comparable to the average duration of viral shedding in immunocompetent patients as reported by To et al¹⁴ and Xu et al,²⁵ which were 20 and 17 days from diagnosis, respectively. Notably, both of our patients had continued tacrolimus, which has demonstrated inhibitory effect on SARS-CoV viral replication in vitro.²⁶ Prolonged viral shedding has additionally been associated with male gender,²⁵ which may explain the increased disease severity and mortality observed in men. Women have been hypothesized to have lower susceptibility to severe COVID-19 disease due to lower viral load, less inflammation, and production of higher antibody levels that remain in circulation longer compared to men.²⁷ However, to what extent IgG antibodies against SARS-CoV-2 can confer protective immunity remains an area of intense research at this time.

The contribution of hydroxychloroquine on our patients' clinical course is uncertain. More data are needed to help draw conclusion regarding the usefulness of hydroxychloroquine in treatment of SARS-CoV-2 in transplant recipients.

In conclusion, the successful management of SARS-CoV-2 infection in kidney transplant recipients requires careful titration of immunosuppression to allow an adequate host viral immune response while maintaining adequate rejection prophylaxis. The availability of serological testing in addition to RT-PCR may be helpful in achieving this delicate balance.

CONFLICT OF INTEREST

We have no conflict of interest or source of funding to disclose.

AUTHORS CONTRIBUTIONS

AXW, SB, and CRL conceived the study and drafted, edited, and reviewed the manuscript. OQC and DYH edited and reviewed the manuscript.

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