

Persistent viral shedding despite seroconversion in a kidney transplant recipient with severe extrapulmonary COVID-19

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SUMMARY

Renal transplant (RT) recipients are at increased risk for infectious complications. The clinical course of COVID-19 has been described in several RT recipients with varying clinical outcomes. Most present with pulmonary manifestations, however extrapulmonary presentations are not uncommon. Also, the timing and efficacy of seroconversion in transplant recipients is not well known. This report describes the duration of viral shedding and timing of seroconversion in a young adult RT recipient with COVID-19 who presented with severe diarrhoea and acute kidney injury requiring dialysis. She developed anti-SARS-CoV-2 IgG antibody after 5 weeks despite persistently shedding the virus in the nasopharynx until 6 weeks after symptom onset. Further studies are needed to determine if immunosuppressed patients have prolonged viral shedding and are still contagious despite seroconversion.

BACKGROUND

SARS-CoV-2 is a novel coronavirus that causes COVID-19. Reports on clinical presentation and course of COVID-19 in renal transplant (RT) recipients continue to emerge. Husain *et al* studied early outcomes in 41 RT recipients with COVID-19 who presented with respiratory symptoms, majority had symptomatic resolution without hospitalisation.¹ In regards to acute kidney injury (AKI), a systematic review and meta-analysis of 20 studies demonstrated an AKI incidence of 8.9% among 6945 patients with COVID-19; only two studies reported data on RT recipients.² Another study showed that 20% of RT recipients had diarrhoea at the time of presentation.³ There is limited data on the immunological response to SARS-CoV-2 infection in immunosuppressed RT recipients. The duration of viral shedding, onset of seroconversion and whether the antibodies confer immunity in immunosuppressed patients are unclear. This report describes a 20-year-old RT recipient with severe diarrhoea and AKI as presenting features of COVID-19 who seroconverted after 5 weeks while still persistently shedding the virus for 6 weeks after the onset of symptoms.

CASE PRESENTATION

A 20-year-old woman underwent a pre-emptive deceased donor RT 4 years ago for end-stage renal disease secondary to dysplastic kidneys. She presented to the intensive care unit with 2-week history of watery diarrhoea, dry cough, intermittent

fever, fatigue and loss of appetite. She had been diagnosed with COVID-19 at the local Department of Health by nasopharyngeal (NP) swab PCR a week after symptom onset. The patient and her mother, who also tested positive for SARS-CoV-2, quarantined at home. There was no history of travel. She did not have nasal congestion, shortness of breath or chest pain. Urine output was normal.

Induction immunosuppression (IS) was done with Thymoglobulin and maintenance IS consisted of tacrolimus, myfortic and prednisone. Two years post RT, the patient had an episode of mixed cellular and antibody rejection and treated with pulse steroid, plasmapheresis, intravenous immunoglobulin (Ig) and Rituximab. Since then, her baseline serum creatinine had been 2.4–2.6 mg/dL. Repeat allograft biopsy had shown severe interstitial fibrosis and tubular atrophy. To prevent further fibrosis, tacrolimus was changed to sirolimus. At the time of COVID-19 presentation, her IS medications consisted of sirolimus 3 mg daily, myfortic 360 mg two times per day and prednisone 10 mg daily.

Initial vital signs revealed oral temperature 37°C, respiration 22 per minute, oxygen saturation 98% on room air, pulse 116 per minute and blood pressure 114/60 mm Hg. Neck and throat examination was unremarkable. Chest examination showed no laboured breathing, reduced air entry, retractions or wheezing. Rest of the examination was normal.

INVESTIGATIONS

Renal function test showed serum creatinine 13 mg/dL, blood urea nitrogen 130 mg/dL, sodium 124 mmol/L, potassium 3.3 mmol/L, chloride 89 mmol/L and bicarbonate 6 mmol/L. White cell count was $13.8 \times 10^9/L$, haemoglobin 12.8 gm/dL and platelet count $223 \times 10^3/mm^3$ with differential of neutrophil 87.7%, lymphocyte 0.4%, monocyte 11.1%, absolute lymphocyte count (ALC) 60/μL and absolute neutrophil count 12 100/μL. Serum C-reactive protein, procalcitonin, ferritin, haptoglobin and lactate dehydrogenase were elevated. There was IgM and IgG hypogammaglobulinaemia.

Repeat NP swab SARS-CoV-2 PCR showed persistent positivity (2 weeks after the symptom onset). Coronavirus (229E, HKU1, NL63 and OC43), influenza A, B, H1-2009 and parainfluenza were not detected. Serum Epstein-Barr virus, cytomegalovirus and BK virus DNA PCR were negative. HIV was negative. Urinalysis showed baseline 2+



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proteinuria, no microscopic haematuria, negative nitrites and leucocytes. Blood and urine cultures were negative. Chest X-ray was normal. Allograft sonogram showed echogenic transplant kidney.

TREATMENT

Emergent haemodialysis was initiated. The patient remained on room air without the need of supplemental oxygen. She had persistent severe diarrhoea and developed an open gluteal wound requiring multiple debridements. Stool PCR was negative for multiple pathogens but not tested for SARS-CoV-2. Due to the concern of delayed wound healing, sirolimus was switched to tacrolimus. Myfortic was discontinued due to active infection and persistent diarrhoea. No antiviral therapies were required. Haemodialysis was discontinued after a few days. Urine output remained normal.

OUTCOME AND FOLLOW-UP

The NP swab PCR was persistently positive at 3 and 4 weeks. SARS-CoV-2 IgG antibody against nucleocapsid (N) protein at 3 weeks was negative (qualitative chemiluminescent microparticle immunoassay, cut-off index ≥ 1.4 , sensitivity 100%, specificity 99.6 %, Abbott Laboratories, Illinois, USA). At discharge (2 weeks after admission, 4 weeks after symptom onset), diarrhoea had resolved and myfortic was restarted. Serum creatinine was 3.7 mg/dL and hypogammaglobulinaemia resolved without the need for replacement Ig therapy. Five weeks after symptom onset, a repeat IgG antibody test returned positive (N protein, index 5.60), but the SARS-CoV-2 NP PCR was also still positive until a week later. Antibody neutralisation test was not performed. At 7 weeks, the NP swab PCR was negative. T-lymphocyte and B-lymphocyte immunophenotyping by flow cytometry showed cluster of differentiation (CD) 19 and CD20 count 0 cells/ μ L, CD4 344 cells/ μ L and CD8 202 cells/ μ L. Peripheral lymphopaenia persisted with 4% lymphocytes and ALC 900 cells/ μ L. Most recent outpatient follow-up at 9 weeks showed serum creatinine of 2.4 mg/dL, and positive IgG antibody (N protein, index 5.59). Baseline proteinuria persisted.

DISCUSSION

Among the four structural SARS-CoV-2 proteins, the N and spike (S) proteins are considered the main immunogens.⁴ Most of the currently available serological assays test IgG antibodies against the N and S protein, with the IgG levels correlating with the virus neutralisation titre.⁵ The IgG antibodies against the N protein may have higher sensitivity, specificity and longer persistence than other structural proteins.⁶ Most patients with COVID-19 seroconvert for IgM and IgG by 2 weeks after disease onset.⁷ Xiao *et al*⁸ demonstrated that IgM antibodies appear early, and last more than a month before starting to decline; IgG appears later but persists longer. Wölfel *et al*⁹ in their study of nine non-immunocompromised patients with COVID-19 showed that the IgM and IgG seroconversion against S protein occurred after day 7 in half of the patients and after day 14 in all patients. However, positive viral load persisted for up to 2 weeks in the NP or throat swabs and up to 3 weeks in sputum and stool specimens. All patients showed detectable neutralising antibodies but the titres did not correlate with clinical courses.

There is very limited data available on duration of viral shedding and serological response to COVID-19 in immunosuppressed patients. Among non-RT immunosuppressed patients, Bollo *et al*¹⁰ described two adults with multiple sclerosis who developed anti-N and anti-S IgG 3 to 5 weeks after the diagnosis

of COVID-19. Wang *et al*¹¹ described two adult RT recipients with COVID-19, both presented with hypoxia, pneumonia, normal allograft function and received hydroxychloroquine. Maintenance IS consisted of tacrolimus, mycophenolate (MMF) and steroid. MMF was held in both patients. One patient had a negative NP swab PCR 30 days after symptom onset followed by development of IgM and IgG antibodies to the spike receptor-binding protein (S-RBD) on day 36. Another patient had seroconverted at day 19 (IgM and IgG to the S-RBD) but still had positive NP swab PCR; 2 weeks later the NP PCR was negative. The second patient's serological profile is similar to our case except that our patient had extrapulmonary presentation, did not receive hydroxychloroquine, had delayed seroconversion and prolonged viral shedding.

In immunosuppressed patients, the duration of presence of IgG antibodies postinfection is not well known. Also, whether these IgG antibodies confer protective immunity is not clear.¹² Reinfection of SARS-CoV-2 was recently reported in a 33-year-old non-immunosuppressed patient who had asymptomatic positive NP swab PCR four and a half months after a symptomatic infection.¹³ The patient did not develop IgG antibody until 5 days after the second infection. Therefore, reinfection can occur in those who do not develop adequate IgG antibodies. To date, and to the best of our knowledge, reinfection has not been described in transplant recipients. Also, as in our case, these antibodies do not exclude recently infected patients who might still be shedding the virus but may not necessarily be contagious. Other potential challenges the immunocompromised patients with COVID-19 may face are that they may have delayed and/or suboptimal antibody response. Indeed, IS can reduce the vaccine-specific antibody response in RT recipients after influenza vaccination.¹⁴ Whether this is also true for SARS-CoV-2 infection is not known at this time.

The role of T and B lymphocytes in the pathogenesis of COVID-19 is unclear. Besides being antibody-producing cells, B cells also act as antigen-presenting cells to T lymphocytes, thereby helping to generate an effective immune response. Hence, serological response to viral infection is considered one of the critical steps for protection against reinfection. Interestingly, the immunophenotyping of the peripheral lymphocytes after seroconversion in our patient showed total absence of B cell markers, CD19 and CD20. Other B cell markers, such as CD24, CD27, CD38, surface immunoglobulins and plasma cell markers were not tested. This patient had received a single dose of Rituximab (375 mg/m²/dose) 2 years earlier. The peripheral B cells start reappearing about 6 months after administration of Rituximab.¹⁵ However, in the setting of IS, the B cell depletion may persist longer. Whether this prolonged B cell depletion is beneficial or disadvantageous in COVID-19 is not clear. In some conditions, such as rheumatoid arthritis and systemic lupus erythematosus, repeated and prolonged B cell depletion has been shown to be efficacious in controlling the disease.¹⁶ With regards to T lymphocytes, SARS-CoV-2 S-reactive and N-reactive CD4⁺ and CD8⁺ T cells have been demonstrated in convalescent plasma of patients who had COVID-19.¹⁷ Hence, in addition to the antibodies, cellular immunity mediated by T cells may also play a critical role for recovery. Whether T cells solely can provide immunity in absence of neutralising antibodies after COVID-19 is an interesting topic and will need to be studied further.

Both IS and viral infections are known to cause lymphopaenia. Our patient had absolute lymphopaenia at the time of presentation, which was still persistent after 7 weeks. Another interesting presentation was IgM and IgG hypogammaglobulinaemia.

A fatal case of COVID-19 in a patient with common variable immunodeficiency with severe hypogammaglobulinaemia has been reported.¹⁸

Extrapulmonary manifestations are common in COVID-19. Sharma *et al*¹⁹ described severe AKI requiring dialysis in eight non-transplant patients with COVID-19; majority had acute tubular necrosis (ATN) but the immunohistochemical staining for SARS-CoV-2 was negative in the biopsy samples. We did not perform a renal biopsy in our patient but the rapid renal recovery suggested ATN as a probable cause of her AKI. Direct viral infection, cytokine-mediated injury and ischaemic/hypoxic/hypovolaemic injury are some of the possible mechanisms of AKI in COVID-19. An International Severe Acute Respiratory and emerging Infections Consortium (ISARIC) WHO Clinical Characterisation Protocol UK (CCP-UK) study of 20 133 hospitalised patients with COVID-19 showed that enteric symptoms including diarrhoea were the presenting features in 29%, in association with respiratory symptoms; 4% presented only with enteric symptoms.²⁰ In RT recipients, the incidence of diarrhoea as a presenting feature appears to be similar.³

Limitations of our report include unavailability of the quantitative viral load in the NP swab specimen, and measurement of the viral load in the stool; IgM serology was not tested, IgG tested was against the N protein only and the neutralisation test was not done. Future studies comparing the serological response and viral shedding profile between immunosuppressed patients with COVID-19 presenting with pulmonary versus extrapulmonary symptoms are needed. Also, whether there is a difference in timing of antibody production to S versus N protein and their efficacy with regards to providing immunity for future infection needs to be examined.

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Patient's perspective

I was really worried when I was diagnosed with COVID-19 given that I have a kidney transplant. I knew that I am immunocompromised as I was taking the anti-rejection medications. However, I was very surprised because besides dry cough, I had no other respiratory symptoms. I always thought that in COVID-19, people get symptoms such as runny nose, difficulty breathing, persistent fever etc. My mother did have cough, runny nose, some difficulty breathing but I had none. As my physicians told me in the hospital that diarrhea and kidney failure could be the presentations of COVID-19, that was a big learning point for me. I had a very difficult clinical course in the intensive care unit due to severe diarrhea and the gluteal wound for which I had to be on a fecal management system. I had to go to the operating room several times for the wound debridement. Also, I had never been on dialysis before the transplant and that when my physicians told me I need a dialysis, that was very scary. Fortunately, my physicians and nurses were very diligent and caring, and hence I overcame a rough time and finally my kidney function was back to baseline. I was able to go home in two weeks despite the challenges I underwent.

Learning points

- ▶ COVID-19 can present with pure extrapulmonary symptoms such as diarrhoea and acute kidney injury.
- ▶ Prolonged SARS-CoV-2 viral shedding can occur in immunocompromised patients such as renal transplant recipients.
- ▶ Antibodies against SARS-CoV-2 can develop even before the nucleic acid test (PCR) turns negative.
- ▶ Transient IgM and IgG hypogammaglobulinaemia and prolonged lymphopaenia can occur in immunosuppressed patients with COVID-19.

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