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Prolonged SARS-CoV-2 shedding and mild course of COVID-19 in a patient after recent heart transplantation

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In the coronavirus disease 2019 (COVID-19) pandemic, organ transplant recipients are considered to be at high risk for an unfavorable outcome. However, in particular the role of immunosuppression in patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains undetermined. Here, we present a 62-year-old male COVID-19 patient with recent heart transplantation who developed only mild symptoms, but had prolonged virus shedding, and summarize the available data on COVID-19 in cardiac allograft recipients. Initially the patient presented with a transient episode of fever and sore throat but no other symptoms, in particular no cough or dyspnea at rest. After diagnosis, immunosuppression was continued unchanged. On day 7, his temperature increased again with concurrent mild rise of C-reactive protein, IL-6, and pro-B-type natriuretic peptide levels. Hydroxychloroquine was started and continued for 7 days. While the patient no longer had clinical symptoms 20 days after initial presentation, virus culture of throat swabs on days 18 and 21 confirmed active virus replication and SARS-CoV-2 PCR remained positive on day 35 with copy numbers similar to the onset of infection. In conclusion, the immunosuppression regimen in transplant recipients with mild COVID-19-associated symptoms may be continued unchanged. However, it may contribute to delayed virus polymerase chain reaction conversion and thus possible prolonged infectivity.

**KEYWORDS**
clinical research/practice, heart disease, heart transplantation/cardiology, immunosuppressant, immunosuppression/immune modulation, infection and infectious agents—viral, infectious disease
In the emergence of the corona virus disease 2019 (COVID-19) pandemic, organ transplant recipients require particular consideration. COVID-19 is typically associated with lung injury, triggering a hyperinflammatory response involving myeloid cells and T cells and excessive production of inflammatory cytokines. It has been hypothesized that immunosuppression could increase susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in transplanted patients. On the other hand, the anti-inflammatory effects of immunosuppression might modify presentation, course, and outcome of COVID-19.

Since many patients with COVID-19 develop cardiovascular complications, heart transplant recipients may be at particular risk. Angiotensin-converting enzyme 2, which has been identified as the SARS-CoV-2 cell entry receptor, is expressed in cardiac myocytes and virus particles have been localized in myocardial biopsy from a patient with COVID-19 complicated by myocarditis. Here, we report a mild course of SARS-CoV-2 infection with prolonged virus persistence in a patient only 5 months after heart transplantation.

The 62-year-old male patient underwent heart transplantation on November 2, 2019 for arrhythmogenic cardiomyopathy of the right ventricle. After transplantation, he remained hospitalized due to a series of complications including pneumonia and acute respiratory distress syndrome (ARDS). He required 56 days of mechanical ventilation and was in need of intermittent renal replacement therapy. Echocardiography revealed a left ventricular ejection fraction of 55% with no signs of transplant rejection. His immunosuppression regimen consisted of cyclosporine A (target range 135 ± 30 ng/mL), mycophenolate mofetil 500 mg b.i.d., and prednisone 10 mg q.d. No anti-lymphocyte globulins had been used as induction therapy. Blood count revealed anemia and leukopenia, the latter likely being caused by immunosuppressive medication, yet not improving significantly under dose reduction. He received cotrimoxazole and due to cytomegalovirus (CMV) high-risk constellation (D + R−), ganciclovir had been administered for 4 months after transplantation and was then switched to valganciclovir prophylaxis. Concurrent medication did not include angiotensin-converting enzyme inhibitors or angiotensin receptor blockers.

On March 13, 2020 (day 1), the patient developed fever (39.9°C), tachycardia (105 bpm), and a sore throat. Polymerase chain reaction (PCR) from throat swab revealed SARS-CoV-2 infection. Body temperature quickly normalized within the first 12 hours. Blood oxygen saturation levels remained stable in the range of 96%-100% without oxygen supplementation at a respiratory rate of 16 breaths per minute. Besides mild rhinorrhea and impaired exercise capacity, the patient showed no other symptoms, in particular no cough or dyspnea at rest. On day 7, a second increase in temperature up to 38.4°C was observed, which resolved spontaneously. This episode went along with a mild rise and peak of C-reactive protein (CRP), IL-6, and pro-B-type natriuretic peptide (proBNP) levels and lymphopenia (Figure 1A-C). A computed tomography scan showed regressive postinflammatory alterations after bacterial pneumonia and ARDS, but no clear signs of COVID-19 pneumonia or bacterial superinfection (Figure 1D). Procalcitonin levels remained low and blood cultures showed no bacterial or fungal growth. We decided to administer hydroxychloroquine (loading dose 400 mg b.i.d. followed by 200 mg b.i.d.) from day 7 to 14. Remarkably, while the patient was free of any marginal residual clinical symptom since day 20, SARS-CoV-2 PCR was positive on days 1, 5, 7, 11, 18, 21, 25, 28, 33, and still on day 35. Concurrent with the second onset of fever we observed an increased viral load after day 7 that slowly returned to the level of infection onset. Whereas the patient already was asymptomatic, virus culture on days 18 and 21 still confirmed active virus replication (Figure 1E).

As proBNP levels increased and decreased simultaneously with the inflammation parameters, we considered a COVID-19-related myocardial infection, yet did not confirm it by myocardial biopsy. No signs of clinical deterioration, notably no signs of cardiorespiratory impairment, were observed. Weaning from hemodialysis was successful (day 1) and urine output and body weight remained stable. Cyclosporine A dose was adjusted several times over the course of infection to achieve a therapeutic range of 135 ± 30 ng/mL (Figure 1F). Otherwise, medication including immunosuppression was continued unchanged except for an increase of prednisone dose to 50 mg for 3 days and 25 mg for another 3 days from day 14 on for treatment of acute gout in the left knee.

Here, we report a case of mild nosocomial COVID-19 in a heart transplant recipient. Though the patient had been considered to be at high risk due to immunosuppression and a complicated course after recent transplant surgery, including respiratory and renal failure, he showed only mild symptoms. Increase in inflammation markers at day 7 was accompanied by a transient rise in proBNP; however, no clinical signs of cardiorespiratory deterioration were observed during the entire course of infection, and increase in proBNP might also have been associated with weaning from hemodialysis. Our experiences in this case underscore the particularities in management of COVID-19 in heart transplant recipients.

Few registries or case series describe course and management of COVID-19 in heart transplant recipients. In a Chinese registry, upper airway infection was reported in 4 out of 87 cardiac allograft recipients, but none of them tested positive for SARS-CoV-2. Among 803 heart transplant recipients from New York (USA), retrospective analysis revealed 28 cases of COVID-19. In total, we have identified
54 cases of COVID-19 in heart transplant recipients from registries or case reports, with full relatable data available for 38 patients (Table 1).\textsuperscript{9-16} To date, out of these evaluable cases including our case reported here, 33 (84.6%) have been hospitalized; 9 of them required invasive ventilation. In total, 24 patients (61.5%) had recovered from infection or were discharged from the hospital at the time of reporting, while 10 patients had died (Table 1).\textsuperscript{9-11,13-16}

As non-CMV infections are the leading cause of death after recent heart transplantation,\textsuperscript{17} reducing immunosuppressive drugs has been discussed to improve outcomes in COVID-19. In fact, in 71.8% of patients with COVID-19 after heart transplant, immunosuppressive agents have been (partially) discontinued or reduced in dose (Table 1), thus potentially increasing the risk of organ rejection.\textsuperscript{9-16} On the other hand, immunosuppressive therapies have been suggested to prevent organ damage caused by hyperinflammation,\textsuperscript{1,3,18} and it has been shown that tacrolimus and a nonimmunosuppressive derivative of cyclosporin A inhibited replication of SARS-CoV-1 and thus possibly decreased viral load.\textsuperscript{19} In our case, continuation of the immunosuppressant regimen was associated with a mild course of COVID-19, though we observed a transient increase in CRP and IL-6. However, retrospective analysis is prone to bias since treating physicians may tend to discontinue immunosuppression in patients presenting with more severe symptoms. Thus, potential benefits of continued immunosuppression require prospective evaluation and need to be carefully balanced against the risk of bacterial superinfection.

Importantly, it has been hypothesized earlier that immunosuppression may impair viral clearance.\textsuperscript{2,20} However, no data are available from heart transplant recipients. We show here that under continued immunosuppression, SARS-CoV-2 was still detectable by reverse transcription polymerase chain reaction (RT-PCR) from oropharyngeal swab up to day 35 of infection. Virus culture performed from throat swab on days 18 and 21 confirmed persistent active virus replication. In contrast to our case, SARS-CoV-2 copy numbers

\begin{figure}
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\caption{Course of COVID-19 in a heart transplant recipient. Leukocyte and lymphocyte count (A), levels of CRP and IL-6 (B), proBNP, and creatinine (C) were followed during the course of disease. Chest computed tomography during SARS-CoV-2 infection (7 d after symptom onset) revealed residues of previous bacterial pneumonia (111 d before symptom onset) but no typical signs of COVID-19 (D). SARS-CoV-2 was detected in throat swabs by RT-PCR (quantity shown as log_{10} copies/mL) and by positive virus culture (dashed black lines) before and after hydroxychloroquine treatment (blue area, E). Administered daily doses (solid black line), serum levels (blue discs), and target range (blue area) of CyA are indicated (F). Dashed red lines indicated date of first symptoms and detection of SARS-CoV-2 in throat swab in each panel. COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CyA, cyclosporine A; IL-6, interleukin 6; proBNP, pro-B-type natriuretic peptide; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2 [Color figure can be viewed at wileyonlinelibrary.com]}
\end{figure}
| TABLE 1 | Summary of the available information on heart transplant recipients with COVID-19 |
|---------|---------------------------------------------------------------------|
|         | Latif et al⁹ | Fernández-Ruiz et al¹³ | Mathies et al¹¹ | Pereira et al¹² | Li et al¹⁴ | Holzhauser et al¹⁵ | Hsu et al¹⁰ | Hoek et al¹⁶ | Decker et al | Total cases/ evaluable cases (%) |
| Total number | 28 4 | 1 12 | 2 2 | 1 4 | 1 55 |
| Single organ (heart) | 28 4 | 1 9 | 2 2 | 0 3 | 1 50/ 55 (90.9) |
| Dual organ (heart and kidney) | 0 0 | 0 0 | 0 0 | 1 1 | 0 5/ 55 (9.1) |
| Age, y | 64 (median) | 64; 63; 38 | n/a | 51; 43 | 59; 75 | 39 | 75; 51 | 62 | 62.5 (median) |
| Male | 22 4 | 1 n/a | 2 1 | 1 3 | 1 35/ 43 (81.4) |
| Time posttransplant, y | 8.6 (median) | 13.8; 10.0; 77 | n/a | 16.2; 2.6 | 8; 20 | 3 | 21; 10; 6 | 0.4 | 10 (median) |
| Maintenance immunosuppression | | | | | | | | | |
| Tacrolimus | 22 1 | 0 n/a | 2 1 | 1 3 | 0 30/ 43 (69.8) |
| Cyclosporine | 5 3 | 0 n/a | 0 1 | 0 1 | 1 11/ 43 (25.6) |
| Mycophenolate mofetil/ mycophenolic acid | 19 4 | 1 n/a | 2 2 | 1 2 | 1 32/ 43 (74.4) |
| Proliferation signal inhibitor | 5 0 | 1 n/a | 0 0 | 0 1 | 0 7/ 43 (16.3) |
| Prednisone | 19 4 | 0 n/a | 0 0 | 1 2 | 1 27/ 43 (62.8) |
| No. of immunosuppressive medications | 1 3 | 0 | 0 n/a | 0 0 | 0 0 | 0 3/ 43 (7.0) |
| 2 | 8 0 | 1 | n/a | 2 2 | 0 3 | 0 16/ 43 (37.2) |
| 3 | 16 4 | 0 | n/a | 0 0 | 1 1 | 1 23/ 43 (53.5) |
| 4 | 1 0 | 0 | n/a | 0 0 | 0 0 | 0 1/ 43 (2.3) |
| Treatment of COVID-19 (antiviral or immunomodulatory therapy) | | | | | | | | | |
| Total number | 23 4 | 1 n/a | 1 2 | 1 3 | 1 36/ 43 (83.7) |
| Hydroxychloroquine | 18 4 | 1 n/a | 0 2 | 1 3 | 1 30/ 43 (69.8) |
| Lopinavir/ritonavir | 0 2 | 0 | 0 1 | 0 0 | 0 3/ 55 (5.5) |
| Remdesivir/placebo | 0 0 | 0 n/a | 0 0 | 1 0 | 0 1/ 43 (2.3) |
| Umifenovir | 0 0 | 0 | 0 1 | 0 0 | 0 1/ 55 (2.3) |
| Oseltamivir | 0 0 | 0 | 0 1 | 0 0 | 0 1/ 55 (2.3) |
| Ribavirin | 0 0 | 0 | 0 1 | 0 0 | 0 1/ 55 (2.3) |
| Interferon β | 0 1 | 0 | 0 0 | 0 0 | 0 1/ 55 (2.3) |
| Ganciclovir | 0 0 | 1 | 0 2 | 0 0 | 0 3/ 55 (5.5) |

(Continues)
| Reduction of immunosuppression | Total number | Dose reduction | At least 1 drug discontinued |
|-------------------------------|--------------|----------------|-----------------------------|
| Tocilizumab                   | 6            | 0              | 0                           |
| Bolus glucocorticoids         | 8            | 0              | 0                           |
| Intravenous gamma globulin    | 0            | 0              | 0                           |
| Total cases / evaluable cases (%) | 8/ 43 (18.6) | 11/ 43 (25.6) | 2/ 55 (3.6) |

| Outcome | No. of patients hospitalized | Need for oxygen supply | Invasive mechanical ventilation | Discharged/recovered at time of reporting | Still hospitalized at time of reporting | Overall mortality at time of reporting |
|---------|-------------------------------|------------------------|--------------------------------|------------------------------------------|---------------------------------------|---------------------------------------|
|         | 22                            | 1                      | 0                              | 17                                       | 4                                    | 7                                    |
| Abbreviations: COVID-19, coronavirus disease 2019; n/a, data not available for this specific subcohort.
showed an exponential decay in immunocompetent patients. In a cohort of 56 patients, the rate of SARS-CoV-2 PCR-positivity was 5.4 and 0.6% at 5 and 6 weeks after symptom onset, respectively. In a Chinese study with consecutive sampling of 49 hospitalized patients every 3 days for 4 weeks, time to clear SARS-CoV-2 was estimated in ≈85% of mild cases at day 35 or 95% at day 46 after illness onset. Notably, in severe cases the estimated time to negativity (95%) was 49 days, implying that hospitalized patients with more severe COVID-19 disease manifestations may have prolonged viral shedding.

Thus, our findings of detectable viral RNA in the oropharyngeal swab in a heart transplant patient with only mild symptoms at day 35 may hint at a possible delayed PCR conversion and possible prolonged infectivity of immunosuppressed patients, thus requiring respective isolation measures in this special cohort. However, patients with low copy numbers in quantitative RT-PCR may not be as infectious. Indeed, preliminary data from our laboratory indicate that virus culture is not successful in swabs that have a RT-PCR Ct > 25 (log 5.3 copies/mL), similar to a recently published study from France showing that SARS-CoV-2 Vero cell infectivity was only observed for RT-PCR Ct < 24. Whether this can be used as an indicator of infectivity, especially in immunosuppressed patients, is currently unknown. In our patient, virus culture was not performed at day 35.

The majority (83.7%) of patients with COVID-19 after heart transplant received an antiviral or immunomodulatory therapy directed against SARS-CoV-2 infection, most of them hydroxychloroquine (69.8%, Table 1). In our case, hydroxychloroquine treatment might have contributed to the persistent mild course of disease after the first week of infection; however, this remains uncertain. We observed some variance in cyclosporine A levels during the course of treatment, eventually associated with the recovery of renal function. In another case, tacrolimus had to be stopped due to uncontrollable high levels after treatment with hydroxychloroquine, remdesivir, and lopinavir/ritonavir. Due to multiple potential interactions with immunosuppressive drugs, serum levels should be closely monitored when hydroxychloroquine or antiviral agents, especially lopinavir/ritonavir, are used in heart transplant recipients.

5 | CONCLUSIONS

This report highlights the particularities of COVID-19 in heart transplant recipients. Although the cardiovascular system seems to be a critical target site of SARS-CoV-2 infection, a mild course of COVID-19 is possible even in a high-risk patient after recent heart transplantation. We conclude that in mild symptomatic transplant recipients, an immunosuppression regimen may be continued unchanged unless prospective data recommend otherwise. Importantly, we demonstrate prolonged virus persistence under immunosuppression up to day 35 of infection, implicating that isolation measures for patients with COVID-19 under immunosuppressive therapy may require particular consideration.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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