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Coronavirus Disease 2019 Causing Infection of Transplanted Lung Allograft: A Pitfall of Prolonged Shedding of Severe Acute Respiratory Syndrome Coronavirus-2 Pretransplant

Sadia Z. Shah, MD, MBA; Francisco G. Alvarez, MD; Devang K. Sanghavi, MD; Pablo Moreno Franco, MD; Shahin Isha, MBBS; Christopher P. Marquez, MD; Claudia Libertin, MD; Pramod K. Guru, MBBS, MD; Basar Sareyyupoglu, MD; and Si M. Pham, MD

Abstract

Coronavirus disease 2019 (COVID-19) pandemic has led to considerable morbidity and mortality across the world. Lung transplant is a viable option for a few with COVID-19–related lung disease. Whom and when to transplant has been the major question impacting the transplant community given the novelty of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We describe a pitfall of presumed prolonged shedding of SARS-CoV-2 in a patient with COVID-19 associated acute respiratory distress syndrome leading to COVID-19 pneumonia after lung transplant. This raises concerns that replication-competent SARS-CoV-2 virus can persist for months post-infection and can lead to re-infection of grafts in the future.

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CASE REPORT

A 47-year-old woman with a medical history of gastroesophageal reflux disease, obesity, and rheumatoid arthritis with no known pre-existing lung disease tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on nasopharyngeal swab (NPS), after an upper respiratory tract infection. She had not received a coronavirus disease 2019 (COVID-19) vaccine before infection and was receiving rituximab and infliximab for rheumatoid arthritis. Despite outpatient treatment with azithromycin and steroids, she deteriorated clinically and needed hospitalization on day 19 post-diagnosis for sudden hypoxic respiratory failure. She was initiated on a high-flow nasal cannula with a flow of 40 L/min (FiO2, 100%) along with dexamethasone, remdesivir, tocilizumab, and convalescent plasma (CP) therapy for worsening hypoxia, as well as trimethoprim-sulfamethoxazole for pneumocystis pneumonia prophylaxis. Her anti-spike antibody titer was negative at admission, 106 IU/mL at day 1 following CP therapy, 4.1 IU/mL at day 35, 3.4 IU/mL at day 39, and 47 U/mL at day 69. Chest X-ray images showed bilateral lung infiltrates, suggestive of COVID-19 pneumonia, which worsened on follow-up chest computed tomography (CT) scans showing diffuse and multifocal airway centric ground-glass opacities along with the fibrotic progression of the COVID-19 pneumonia. Due to persistent severe hypoxia with increasing oxygen demands, a lung transplant (LT) evaluation was initiated on day 50 after COVID-19 diagnosis. Although she persistently tested positive for SARS-CoV-2 during transplant evaluation, it was attributed to possible viral shedding, and she was listed

From the Department of Transplantation (S.Z.S., F.G.A.), Department of Critical Care (D.K.S., P.F.-M., S.I., P.K.G.), Department of Lab Medicine and Pathology (C.P.M.), Division of Infectious Disease, Department of Medicine (C.L.), and Department of Cardiothoracic Surgery (B.S., S.M.P.), Mayo Clinic, Jacksonville, FL.

for LT based on the International Society of Heart and Lung Transplant guidelines, which recommends asymptomatic candidates with persistent reverse transcriptase–polymerase chain reaction (RT-PCR) being waitlisted if they are more than 28 days from initial infection, and the risk of mortality is high.[1] Her lung allocation score at the time of listing was 90, and she received a bilateral orthotopic LT on the 74th day after COVID-19 diagnosis. Post-transplant, she required 5 cycles of plasmapheresis, 3 doses of antithymocyte globulin, as well as maintenance immunosuppression with tacrolimus, mycophenolate, and prednisone due to T and B-cell positive crossmatch. The patient's NPS sample, as well as the donor's bronchoalveolar lavage (BAL), were negative for SARS-CoV-2 on the day of the transplant. Although recovering well initially, she developed worsening hypoxic respiratory failure and chest pain 2 weeks after transplant (88 days after COVID-19 diagnosis), requiring re-admission to the intensive care unit. In addition to invasive mechanical ventilation with high positive end-expiratory pressure, 100% FiO2, and inhaled nitric oxide, she also received remdesivir and high-dose methylprednisolone and was rediagnosed with COVID-19 on BAL testing. Her clinical course was also complicated with 2 witnessed events of vomiting and possible aspiration, and her hypoxia progressed to the extent of veno-venous extracorporeal membrane oxygenation (VV-ECMO) requirement on day 100 after initial COVID-19 diagnosis. Despite supportive measures on VV-ECMO for 2.5 months, she died 100 days after LT. The timeline of the case and SARS-CoV-2 test results are shown in Figure 1.

METHODS

SARS-CoV-2 RT-PCR testing was performed on the pre-transplant NPS using the Xpert Xpress SARS-CoV-2 assay system (Cepheid) and on the post-transplant BAL specimen using BioFire Respiratory Panel 2.1 (bioMérieux).[2,3] The BioFire Respiratory Panel 2.1 is a PCR-based multiplexed nucleic acid test, intended for use with the BioFire 2.0 or BioFire Torch Systems for simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in NPS samples obtained from individuals suspected of respiratory tract infections, including COVID-19. We were able to generate genetic sequences from both pre-transplant (day before transplant) and post-transplant specimens (day of COVID-19 diagnosis after transplant) using phylogenetic mapping.

RESULTS

Phylogenetic mapping of the 2 sequences indicated that they were closely related, although the post-transplant viral strain had 10 more mutations than the pre-transplant strain. These results indicate that the virus likely persisted post-transplant and acquired some

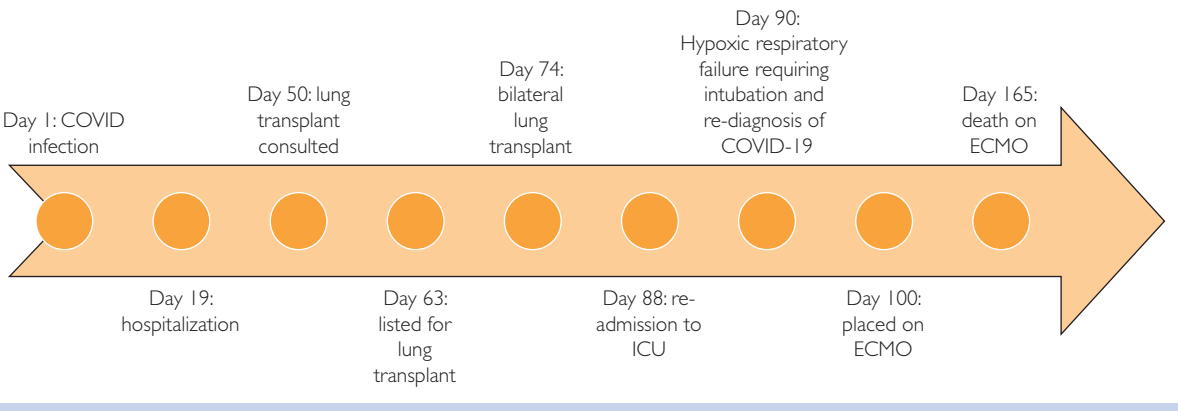

FIGURE 1. Timeline of the case and severe acute respiratory syndrome coronavirus 2 test results. COVID-19, coronavirus disease 2019; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

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additional genomic mutations, which is consistent with a replication-competent virus. The cycle threshold value was 28 on the initial BAL sample but decreased to 14 on follow-up BAL specimens when the patient clinically worsened. Viral cultures were not done due to the unavailability of a Biosafety level-3 (BSL-3) Laboratory. Bronchoscopy with BAL was positive for SARS-CoV-2 and yeast, but no other organisms were identified. A computed tomography scan of the chest with contrast was negative for pulmonary embolism but positive for bilateral ground-glass changes, which later transitioned to consolidations. The timeline of radiographic images after LT, at the time of diagnosis of COVID-19 re-infection, and close to the need for VV-ECMO are shown in Figure 2. Transbronchial biopsies were negative for acute rejection but showed an organizing acute lung injury pattern. Human leukocyte antigen and non-human leukocyte antigen donor-specific antibodies were also negative, making it unlikely to be an antibody-mediated rejection. Postmortem autopsy showed acute organizing diffuse alveolar damage evidenced by the extensive presence of hyalinized material, a morphologic feature for diffuse alveolar damage.

DISCUSSION

Management of patients with COVID-19—related end-stage lung disease has evolved since the start of the COVID-19 pandemic. When evaluating a patient with COVID-19, transplant physicians assess if the damaged lung's can potentially recover by itself over 6 to 8 weeks. If the damaged lung fails to recover, LT evaluation is initiated. However, this timeline may be shorter or longer given the severity of the disease, the likelihood of potential mortality, and the availability of certain resources, such as ECMO. Cypel et al[4] shared their initial experiences regarding when to consider LT for patients with COVID-19 and the importance of patients having a recent negative SARS-CoV-2 RT-PCR result before a LT. Severe acute respiratory syndrome coronavirus 2 RT-PCR testing is highly sensitive but not specific enough to diagnose the infectivity of SARS-CoV-2. It may be positive for 90 days or more after infection, making it difficult to evaluate patients for transplant who are struggling with COVID-19-associated acute respiratory distress syndrome. The Center for Disease Control and Prevention advises that immunocompetent adults should quarantine for 5 days after symptom onset,

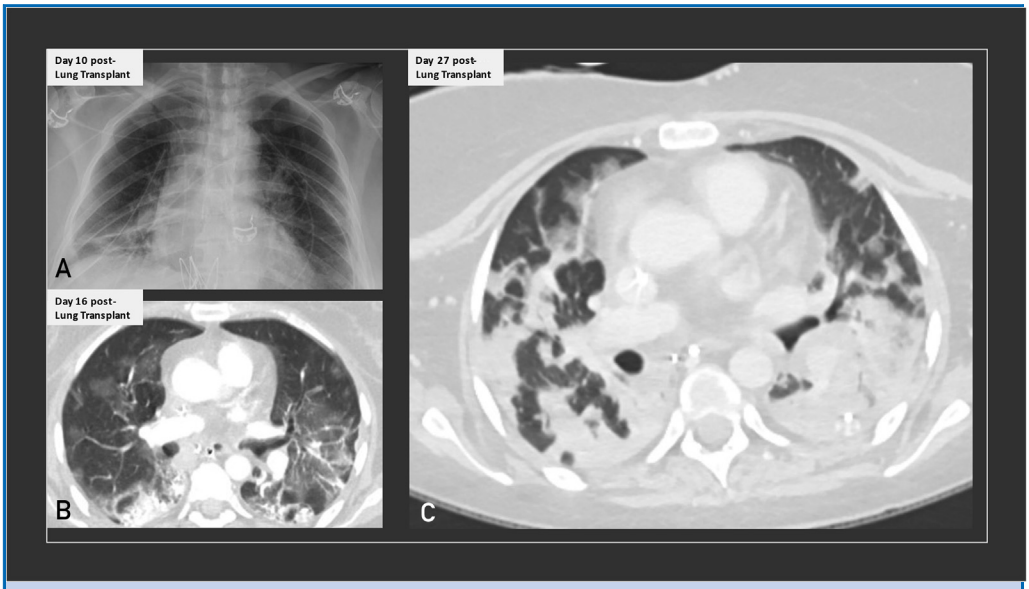

FIGURE 2. Timeline of radiographic progression after lung transplant. A, On day 10 after lung transplant. B, At the time of diagnosis of coronavirus disease 2019 re-infection. C, Close to the need for veno-venous extracorporeal membrane oxygenation.

whereas immunocompromised adults or severely ill patients should quarantine for 20 days after symptom onset (granted no fever within 24 hours and other COVID-19–related symptoms are resolving).[5] Van Kampen et al[6] also described the duration and key determinants of viral shedding in hospitalized patients with COVID-19. In their systemic review and meta-analysis of 79 studies (5340 individuals with SARS-CoV-2 infection), Cevik et al[7] found that the mean duration of SARS-CoV-2 ribonucleic acid (RNA) shedding was 17 days in the upper respiratory tract (95% CI: 15·5-18·6 days; 43 studies including 3229 individuals); 14.6 days in the lower respiratory tract (95% CI: 9.3-20 days; 7 studies including 260 individuals); 17.2 days in stool samples (95% CI: 14.4-20.1 days; 13 studies including 586 individuals); and 16.6 days in serum samples (95% CI: 3.6-29.7 days; 2 studies including 108 individuals). Maximum shedding duration was 83 days in the upper respiratory tract, 59 days in the lower respiratory tract, 60 days in serum samples, and 126 days in stool samples. None of these studies detected a live virus beyond day 9 of illness, despite persistently high viral loads, which were inferred from cycle threshold values.[7] However, questions remain on whether these timelines can be used when assessing patients for LT eligibility and what should be done when a patient is persistently shedding the virus for more than 28 days.

Some patients persistently test positive with SARS-CoV-2 RT-PCR months after their COVID-19 diagnosis and are called long-term viral carriers. Li et al[8] looked at the timeframes of viral shedding in these long-term viral carriers. They found that the median viral RNA shedding was 92 days, and the longest carrying history was 118 days. In their cohort, negative–positive viral RNA-shedding fluctuations were also observed, which could be due to varying phases of viral replication. Infectious SARS-CoV-2 was isolated from sputum in which high-level viral RNA was found, and all 9 full-length genomes of samples obtained 3 months postinfection matched early viral clades, suggesting that these patients persistently carried infectious SARS-CoV-2 and were not re-infected. Moreover, they found that most of the carriers in their cohort were older than 65 years, had mild disease at first diagnosis, and were mostly asymptomatic. However, they had COVID-19 pneumonia-related lesions on the basis of chest CT and neutralizing antibody titer profiles similar to recovered patients.

The review by Binnicker[9] on COVID-19 testing assessing infectivity is summarized in Figure 3. Because SARS-CoV-2 RT-PCR testing cannot differentiate between infectious vs viral shedding, cycle threshold values may be used to determine infectivity of the virus. The cycle threshold value is the number of cycles a sample must be amplified in the laboratory before virus can be detected. A low cycle threshold value is consistent with a higher viral load and infectivity as fewer cycles are needed to detect the virus. Unfortunately, the cycle threshold values are affected by sampling technique and assay variabilities, reducing their reliability. Viral culture is considered the gold standard, but unfortunately, most centers do not have a BSL-3 Laboratory to perform these cultures. Phylogenetic testing can be considered for patients with persistent shedding of the virus, to assess, if it is the same virus or a re-infection with a different strain, but the CT value needs to be low enough to perform this test. Apart from sampling variabilities and negative–positive viral shedding fluctuations influencing the ability to run this test, cost-effectiveness, and availability are other factors to be considered.[9] Whether it is possible to demonstrate persistent, replication-competent (infectious) virus in a patient by the accumulation of mutations, like we did in our patient, needs to be assessed in future studies.

A positive SARS-CoV-2 RT-PCR result after 2 consecutive negative RT-PCR tests after recovery from COVID-19 have been reported and can be attributed to the dead virus, sampling error, or effect of discontinuation of drugs to combat COVID-19.[10-14] Re-infection always remains a concern in these patients, especially the ones with viral shedding for more than 3 months. Falahi et al[10] described that for true re-infection diagnosis, certain criteria must be considered: isolation of the complete genome of the virus (and not only genomic fragments) in the second episode; identification of viral strains that is phylogenetically different in 2 episodes of infection; proof of virus infectivity and cytopathic effect in the second episode, evidenced by virus

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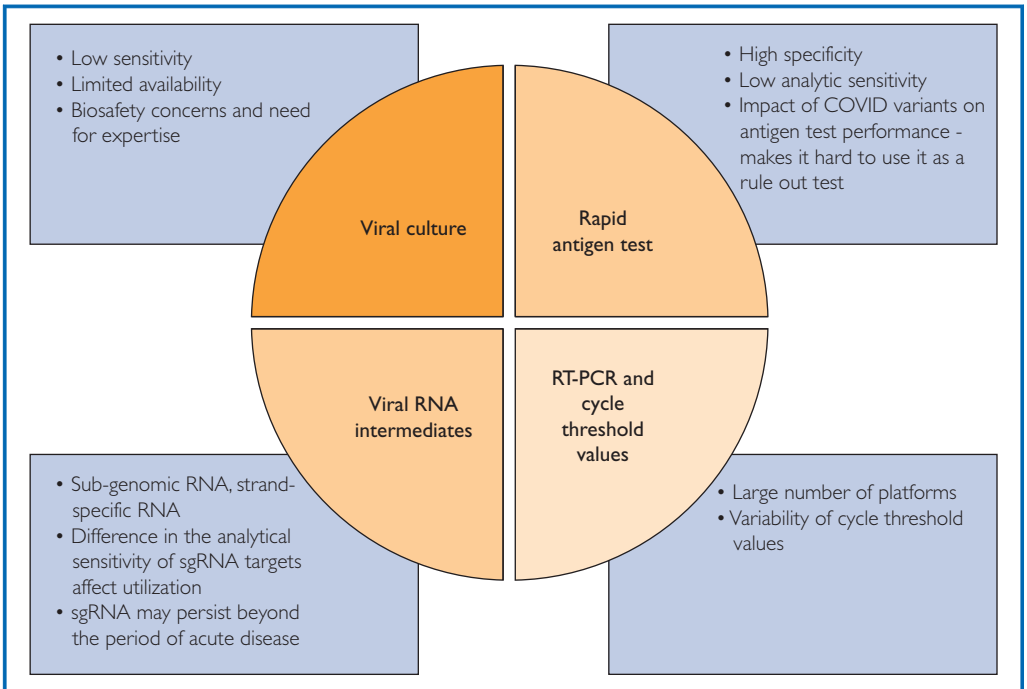

FIGURE 3. Tests to assess infectivity of SARS-CoV-2. COVID-19, coronavirus disease 2019; RNA, ribonucleic acid; RT-PCR, reverse transcription—polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sgRNA, single guide RNA.

culture; investigation of immune responses and their comparison in 2 episodes; and relevant epidemiologic data, such as re-exposure history to a patient with COVID-19 in the second event and a longer time interval between 2 episodes favoring the re-infection hypothesis.[10,13] In our patient, we could not perform viral culture due to BSL-3 unavailability, but the identification of phylogenetically similar strains, proof of viral infectivity with a clinical decline of the patient requiring progression from intubation to requiring VV-ECMO for months, no known exposure history due to strict precautions and testing, evidence of lower cycle threshold value on specimens after an initial higher value and persistence of anti-spike antibody (47 IU/mL) on the day of rediagnosis of SARS-CoV-2 after transplant make re-infection less likely than viral persistence.

This case and the above data have important implications for the LT community, and it should be looked at more closely from an infectivity standpoint. Patients with high panel reactive antibody before transplant or a positive crossmatch peri-transplant who require peri-operative plasmapheresis and induction immunosuppression therapy may be at a higher risk of COVID-19 infection after transplant, but more data are needed to make that determination. Whether plasmapheresis impacts the neutralizing antibodies, needs to be investigated as well. Anti-CD20 agent (such as rituximab) therapy associated with prolonged persistence of SARS-CoV-2 in respiratory samples have also been reported in multiple instances and need further research.[15] Other sources of re-infection can potentially be the nasopharynx and gastrointestinal tract, which harbors the virus longer than the upper or lower respiratory tracts.[7,11] Further studies are also needed to determine whether gastroesophageal reflux disease or aspiration events can lead to infection of lung graft and how we can address such concerns during transplant evaluation.

In conclusion, recurrent COVID-19 infection due to prolonged viral shedding remains

a potential cause of lung graft failure in the immediate post-transplant period. Determination of virus infectivity in the host remains a challenge and need for cautious pre-transplant evaluation remains prudent in these cases.

POTENTIAL COMPETING INTERESTS

The authors report no competing interests.

Abbreviations and Acronyms: **BAL**, bronchoalveolar lavage; **BSL-3**, Biosafety level-3; **COVID-19**, coronavirus disease 2019; **CP**, convalescent plasma; **CT**, computed tomography; **LT**, lung transplant; **NPS**, nasopharyngeal swab; **RT-PCR**, reverse transcriptase—polymerase chain reaction; **SARS-CoV-2**, severe acute respiratory syndrome coronavirus 2; **VV-ECMO**, veno-venous extracorporeal membrane oxygenation

Correspondence: Address to Sadia Z. Shah, Department of Transplantation, Mayo Clinic, 4500 San Pablo Rd S, Jacksonville, FL 32224 (shah.sadia@mayo.edu).

ORCID

Sadia Z. Shah: https://orcid.org/0000-0001-5588-3873; Pablo Moreno Franco: https://orcid.org/0000-0001-9146-5599; Shahin Isha: https://orcid.org/0000-0002-6613-6395; Christopher P. Marquez: https://orcid.org/0000-0003-4244-3259; Pramod K. Guru: https://orcid.org/0000-0001-7695-4407

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