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Persistent SARS-CoV-2 infectivity greater than 50 days in a case series of allogeneic peripheral blood stem cell transplant recipients

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A B S T R A C T
The coronavirus disease 19 (COVID-19) pandemic has infected tens of millions across the world, but there is a significant gap in our understanding about COVID-19 in the hematopoietic stem transplant (HSCT) recipient population. Prolonged viral shedding is frequently observed with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), but studies suggest viral loads decline 10 days after symptom onset. Current CDC guidance suggests that severely ill and immunocompromised hosts are no longer infectious after 20 days from symptom onset. Cycle threshold (Ct) values are inversely proportional to the viral load and are used to detect SARS-CoV-2 RNA concentration. Specimens with reverse transcriptase PCR (RT-PCR) Ct values > 33–34 have been associated with inability to culture virus, and have been used as a surrogate for diminished infectivity. We report two cases of allogeneic peripheral blood stem cell transplant (PBSCT) recipients who had prolonged durations of infectivity with SARS-CoV-2, based on culture positivity and persistently low Ct values for greater than 50 days.

Introduction
The coronavirus disease 19 (COVID-19) pandemic has infected hundreds of millions across the world, but there is a significant gap in our understanding about COVID-19 in the hematopoietic stem transplant (HSCT) recipient population. Prolonged viral shedding is frequently observed with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), but studies suggest viral loads decline 10 days after symptom onset. Cycle threshold (Ct) values are inversely proportional to the viral load and are used to detect SARS-CoV-2 RNA concentration. Specimens with reverse transcriptase PCR (RT-PCR) Ct values > 33–34 have been associated with inability to culture virus, and have been used as a surrogate for diminished infectivity. We report two cases of allogeneic peripheral blood stem cell transplant (PBSCT) recipients who had prolonged durations of infectivity with SARS-CoV-2, based on culture positivity and persistently low Ct values for greater than 50 days.

Case series
Patient 1 is a 26-year-old male with relapsed refractory pre-B cell acute lymphoblastic leukemia who underwent an allogeneic haploidentical PBSCT on April 13, 2020. He was treated with the CALGB 10,403 regimen, followed by vincristine, daunorubicin, PEG asparaginase, and intrathecal methotrexate plus cytarabine. He received central nervous system prophylaxis with intrathecal methotrexate and cytarabine. He then underwent an allogeneic haploidentical PBSCT with a conditioning regimen of busulfan, fludarabine, and cyclophosphamide. Graft versus host disease (GVHD) prophylaxis consisted of post-transplant cyclophosphamide, tacrolimus, and mycophenolate. Mycophenolate was initiated on day 5 post-transplantation and continued for 30 days. His neutrophils engrafted on day 15 post-PBSCT; however, he remained pancytopenic and persistently lymphopenic. His-day 30 post-PBSCT chimerism showed 100% CD3+ and 33% CD3+ donor cells.

On day 38 post-PBSCT, he developed a fever to 38.3°C and a dry cough. He reported abdominal pain and diarrhea the following week. On day 52 post-PBSCT, a nasopharyngeal swab (NP) from the patient

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tested positive for SARS-CoV-2 (N2 Ct = 19) by RT-PCR, using the Xpert Xpress SARS-CoV-2 Assay. His-initial chest radiograph showed bilateral interstitial infiltrates (Fig. 1). He was notably pancytopenic with a CMV viral load of 2091,000 IU/mL. G-CSF was started for leukopenia. GVHD prophylaxis with tacrolimus was discontinued on day 58 post-PBSCT. He was treated with 24 days of ganciclovir and 5 days of remdesivir. He received dexamethasone for 6 days for hypoxemia (The RECOVERY Collaborative Group, 2020). On day 67 post-PBSCT, he developed graft failure (absolute neutrophil count < 0.5 K/μl despite daily G-CSF). He was discharged home with valganciclovir with a CMV viral load of 1330 IU/mL. He remained persistently SARS-CoV-2 RT-PCR positive 20 and 23 days post initial test. After 28 days of isolation, he returned to the outpatient infusion center for biweekly transfusions. His-day 72 post-PBSCT chimerism showed 96% CD33+ and 1% CD3+ donor cells, suggesting progression of the recipient’s lymphocyte reconstitution with an initial loss of donor myeloid engraftment.

Fifty-three days post-initial onset of COVID-19 symptoms, he reported worsening cough and fever up to 39.8°C. Repeat chest radiograph showed worsening infiltrates. Repeat RT-PCR testing 54 days post symptom onset using the same Xpert Xpress SARS-CoV-2 Assay was positive (N2 Ct = 23.2). In addition to a positive RT-PCR result, viral culture was performed off the NP swab collected on day 54 at a biosafety level-3 lab, and it showed the presence of infectious SARS-CoV-2.

He was restarted on remdesivir for 10 days. On day 5 of remdesivir, his fever, cough, and radiographic findings improved, however, repeat RT-PCRs with the Xpert Xpress SARS-CoV-2 Assay were positive at day 59 (N2 Ct= 27) and day 63 of illness onset (N2 Ct=22) (Table 1). His-SARS-CoV-IgG antibody at day 56 post illness onset was negative. On day 86 post illness onset, his RT-PCR Ct value increased to 41.5, deeming him noninfected. He remained pancytopenic with an absolute neutrophil count of 0 despite daily g-CSF. His-day 93 post-PBSCT chimerism showed incalculable CD33+ and 0% CD3+ donor cells. His-bone marrow biopsy on day 113 post-PBSCT showed a hypocellular bone marrow with early regenerative activity and no morphologic evidence of residual leukemic blasts. His-chimerism from the bone marrow showed 63% CD33+ and 0% CD3+donor cells compatible with full recipient lymphocyte reconstitution and progressive loss of his donor myeloid engraftment.

Following loss of his graft, the patient underwent a stem cell boost on day 125 post-PBSCT with minimal change in his pancytopenia. He ultimately required a second allogeneic haploidentical PBSCT, preceded by a regimen of fludarabine, cyclophosphamide and anti-thymocyte globulin. Neutrophils engrafted 12 days post PBSCT. His-post transplant bone marrow chimerism on day 91 post second PBSCT revealed 98.7% CD33+ and 98.7% CD3+ donor cells, indicating near complete donor engraftment. His-bone marrow biopsy showed a hypercellular bone marrow with marked erythroid predominance, reduced myeloid precursors and neutrophils, and no evidence of leukemia.

Our second patient is a 23 year old female with acute myeloid leukemia (AML) who initially received induction chemotherapy with cytarabine and daunorubicin followed by re-induction with mitoxantrone, etoposide and cytarabine. She required salvage chemotherapy with fludarabine, cytarabine, idarubicin, and gemtuzumab ozogamicin due to relapse. She underwent an allogeneic matched related PBSCT, following a conditioning regimen of busulfan and cytoxan. Her neutrophils engrafted on day 12 post-PBSCT. Her day 100 post-PBSCT bone marrow biopsy showed slightly hypercellular bone marrow (50% cellular) with normal dysplasia, mildly increased blasts (approximately 3% of marrow cellularity) and hematogones (10%), compatible with regenerative changes. Because the day 100 post-PBSCT chimerism showed 97% donor cells, 3% recipient cells and high risk of disease, she was started on low dose azacitadine maintenance treatment.

| Table 1 | Results of NP rapid SARS-CoV-2 PCR for patient 1. |
|---------|--------------------------------------------------|
| Treatment day of remdesivir | Rapid SARS-CoV-2 PCR Ct value | Treatment day 0 | Treatment day 6 | Treatment day 10 |
| illness day 15 | Positive | 19.9 | 22.2 | 41.5 |
| illness day 54 | Positive | 22.2 | 27.8 | 22.5 |
| illness day 59 | Positive | 22.5 | 22.5 | 22.5 |
| illness day 63 | Positive | 22.5 | 22.5 | 22.5 |
| illness day 86 | Positive | 22.5 | 22.5 | 22.5 |

Ct values were all reported using Xpert Xpress SARS-CoV-2 Assay. Only N2 values are reported in this case report.
She developed persistent pancytopenia and repeat bone marrow biopsy three months later showed relapsed AML with 55–60% blasts, which were positive for CD15, CD33, CD34, CD45, CD117. She failed reinduction with clofarabine and cytarabine. She was started on salvage chemotherapy with topotecan, vinorelbine, thiopeta, gemcitabine, and dexamethasone. Her hospital course was complicated by cardiogenic shock with an ejection fraction of 15–20%, wide open tricuspid regurgitation, and mild to moderate aortic valve regurgitation. She required mechanical ventilation in the intensive care unit (ICU) and was noted to have bloody secretions. SARS-CoV-2 RT-PCR testing was negative at that time. She developed enterococcus bacteremia, related to a line infection and received 14 days of linezolid. She was also started on isavuconazole for a presumed pulmonary angioinvasive mold infection per her clinical symptoms and imaging. CT chest showed multiple pulmonary nodules with surrounding linear lucencies consistent with an angioinvasive fungal infection. She clinically improved, was subsequently extubated and transferred out of the ICU.

While on the medical ward, she was exposed to a nurse who later tested positive for SARS-CoV-2. The nurse was asymptomatic at the time she took care of the patient but developed symptoms shortly thereafter. As part of the exposure workup given the known presymptomatic and asymptomatic spread with SARS-CoV-2 (Gandhi et al., 2020; Arons et al., 2020; Tong et al., 2020), the patient was tested for SARS-CoV-2 by RT-PCR, using the Xpert Xpress SARS-CoV-2 assay and tested positive. She was asymptomatic at the time of testing. Because of her severely immunocompromised state and high risk of complications, she was treated with 5 days of Remdesivir. She remained asymptomatic throughout the course of treatment. On day 24 post diagnosis, repeat RT-PCR using the same Xpert Xpress SARS-CoV-2 methodology, revealed a Ct of 17.1. On day 41 post diagnosis, patient received convalescent plasma to aid her immune response and clearance of the virus. At 49 days post diagnosis, her RT-PCR Ct value increased to 28.9. At day 56 post diagnosis, her RT-PCR Ct value increased to 34.1, deeming her noninfectious (Table 2).

**Discussion**

SARS-CoV-2 has been recognized to cause prolonged viral shedding in the nasopharynx, but previous studies have established that the viral load rapidly decreases after day 10 of symptoms (Liu et al., Aug). Multiple studies have indicated that transmission occurs early, predominantly in the first week of symptoms (Cheng et al., 1; Wolfel et al., 2020). Understanding the transmission pattern in relation to levels of viral shedding has guided infection control principles in managing the SARS-CoV-2 pandemic (Atkinson and Petersen, 2020; Hei et al., 2020). Current CDC guidance recommends using a symptom-based strategy for discontinuing transmission-based precautions (CDC 2020). For patients with mild to moderate illness who are not severely immunocompromised, transmission-based precautions can be discontinued after 10 days from symptom onset if the patient has been afebrile for 24 hours and if the symptoms have improved. A duration of 20 days has been proposed for patients with critical illness or for patients who are severely immunocompromised. Previous studies have proposed that the presence of neutralizing antibody is associated with the presence of non-infectious SARS-CoV-2 and decreased infectivity of persistently positive patients (Van Kampen et al., 2020).

Our first patient is unique in that he acquired SARS-CoV-2 < 40 days post allogeneic PBSC with a naive immune system that was incapable of producing the T cell mediated or neutralizing antibody responses that are fundamental for viral clearance. Repeated testing demonstrated persistently low RT-PCR Ct values (in the 20s) up to 63 days post initial symptom onset with symptom recurrence and culture positivity at day 54 post symptom onset, indicating ongoing infectivity. Cycle thresholds have been used as an indicator of viral load (Yu et al., 2020; Liu et al., 19) and specimens with RT-PCR Ct values greater than 33–34 have been associated with inability to culture virus (La Scola et al., 2020). In the Korean CDC epidemiological investigation for 447 “re-positive” cases, Ct values for 93 samples tested showed 89.5% of the tests had a Ct value >30 and all the cases had neutralizing antibody production. None of these cases contributed to secondary cases (KDCA 19 May 2020). In contrast, our first patient had no detectable SARS-CoV-2 IgG, and his NP SARS-CoV-2 RT-PCR had consistently low N2 Ct values in the 20s. It is important to note that the same platform was used to determine the N2 Ct value as Ct values across different assays can vary in their correlation of detection of SARS-CoV-2 RNA (Moore et al., 2020). Her-recrudescence of symptoms 53 days after his initial symptom onset was likely a direct result of persistently high viral loads and lack of a humoral immune response (evidenced by his negative IgG antibody). In addition, the viral culture performed off his NP specimen from day 54 post symptom onset was positive, indicating viable virus and ongoing infectivity (Huang et al., Aug). Similarly, our second patient continued to have persistently low Ct values in the 20s up to 49 days post diagnosis, indicating high viral loads and infectious SARS-CoV-2 despite a 5 day course of Remdesivir and entirely asymptomatic course of infection (Ballard et al., 2020).

One study looking at 20 immunocompromised patients with hematologic malignancies also found prolonged viral shedding and viable virus for more than 20 days in 3 patients who had received allogeneic PBSC (2 patients) or CAR T-cell therapy (1 patient) (Aydillo et al., 2020). Of note, this study included 8 patients with lymphoma, 4, patients with acute leukemia, 1 with chronic leukemia and 7 with multiple myeloma; 15 of which who were receiving chemotherapy, but only the allogeneic PBSC and CAR-T cell patients had prolonged viral viability beyond 20 days.

During the course of his COVID-19 infection, our first patient rapidly lost his CD3+ donor cells. Although the direct cause of his graft failure is not clear, viral infections, such as CMV, human herpesvirus type 6, and parvovirus have been implicated in graft failure (Mattsson et al., 2008). SARS-CoV-2 is also known to cause lymphopenia, and post-transplant lymphopenia has also been associated with higher risk of graft failure, death, and viral infections (Dujardin et al., 2019). Based on the rapid loss of his myeloid line at the time of his infection, the CMV viremia and SARS-CoV-2 infection were thought to be likely contributors to the loss of his graft.

Both patients posed challenging questions in terms of treatment and duration of SARS-CoV-2 infectivity in the PBSCCT population. Our first patient was treated with two courses of Remdesivir, including a 10 day course the second time. Remdesivir did not appear to decrease the SARS-CoV-2 viral load significantly, although it did help in clearing the patient's symptoms (Wang et al., 2020; Beigel et al., 2020; Goldman et al., 2020; Spinner et al., 2020). In our second patient, convalescent plasma appeared to be an effective adjunctive treatment in helping to clear the virus quickly, noted by the significant weekly increase in Ct values following administration of convalescent plasma (Focosi et al., 2020; Liu et al., 2020; Joyner et al., 2020). Convalescent plasma and monoclonal antibodies may be important treatments for those patients who are incapable of producing a humoral or T cell mediated immune response, especially if prolonged high levels of viral shedding continue to pose a transmission risk to others.

Our first patient was transfusion-dependent, and his infusion center was also attended by other immunocompromised hosts. In the contact tracing that was performed, there was an infusion nurse who was symptomatic with a cough and a fever 5 days after contact with the patient, but she tested negative by NP SARS-CoV-2 RT-PCR. Universal masking precautions and the single patient room for this patient likely facilitated decreased transmission risk, but it is also possible that the exposed nurse
had a false negative RT-PCR test given her corresponding symptoms and known exposure. Current CDC guidelines recommend 20 days as the duration of isolation and infectivity for immunocompromised or critically ill patients, but in allogeneic PBST recipients, this time frame may not be long enough. COVID-19 may also constitute a particularly increased threat of graft failure in the early post HSCT period, and emphasize precaution needs to be encouraged. This possibility of graft failure as well as prolonged infectivity are important arguments to prioritize vaccination of household members of allogeneic PBST recipients. Further exploration using clinical symptoms, Ct values, and culture data to determine duration of infectivity in the HSCT recipient patient population is warranted (Gniazdowski et al., 2020; Ljungman et al., 2020; Waghmare et al., 2020).

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There are no financial disclosures to report.

Declaration of Competing Interest
None.

Patient consent
Written, informed consent was obtained from both patients.

CRediT authorship contribution statement
Alice Han: Conceptualization, Formal analysis, Project administration, Writing - original draft. Tulio E. Rodrigues: Conceptualization, Formal analysis, Writing - review & editing. Eric T. Beck: Data curation, Methodology, Resources, Writing - review & editing. Ryan F. Relich: Data curation, Methodology, Resources, Writing - review & editing. Dioma U. Udeoji: Writing - review & editing. Robert Petrak: Writing - original draft. Vishnu V. Chundi: Writing - review & editing.

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