DOI: 10.1002/jmv.27832

LETTER TO THE EDITOR

JOURNAL OF MEDICAL VIROLOGY WILEY

Persistent high-level shedding of cultivable SARS-CoV-2 Delta virus 33 days after onset of COVID-19 in a hospitalized patient with pneumonia

Dear Editor

The viral dynamics of SARS-CoV-2 infections with non-Delta strains has followed a typical trajectory of viral RNA shedding for a mean duration of 17 days, accompanied by progressive decline in viral load and subsequent virus culture negativity.[1] However, the Delta variant is known to be more contagious, has a longer duration of virologic shedding, and more likely to result in severe illness than other variants of SARS-CoV-2.[2] Here, we report prolonged shedding of SARS-CoV-2 Delta variant (B.1.617.2) for over 29 days in six patients with severe coronavirus disease (COVID-19) admitted to the intensive care unit (ICU) in Australia. One patient (P6) remained culture-positive 33 days after onset of disease (GISAID Accession: EPI_ISL_3874692). These features, including the persistence of high viral load ($C_t < 30$) in these patients, present new uncertainties concerning the safe discharge of patients from isolation into the general inpatient population.

The patients were admitted during a predominantly Delta variant outbreak in July 2021 (winter) to a quaternary referral center in Sydney, Australia. Patients were hospitalized within 7 days of the first SAR-CoV-2 positive swab result (considered as Day 0 here) with symptom onset approximately 1–2 days prior in all cases. Other than P3 (52-year-old male) and P4 (69-year-old male), all patients had comorbidities previously known to be associated with severe disease (Supporting Information: Table 1). Patients P1 (77-year-old male) and P5 (51-year-old male) had type 2 diabetes (T2D); P2 (72-year-old female) had chronic obstructive pulmonary disease, and P6 (56-year-old female) was 22-days posttherapy for multiple myeloma. All patients were unvaccinated for SARS-CoV-2 and experienced respiratory compromise just before or soon (less than 24 h) after admission. Patients received treatment with dexamethasone and tocilizumab (1–8 days from admission) as standard of care, with P2 also receiving a course of remdesivir. All patients developed COVID pneumonitis clinically (reduced oxygen saturation and chest X-ray abnormalities) and required transfer to ICU for ventilatory support. Patient P5 required veno-venous extracorporeal membrane oxygenation (ECMO) for progressive respiratory failure. Patient P6 died 35 days following disease onset and 32 days after ICU admission. Other patients (P1–P5) were discharged a median of 43 days (interquartile range [IQR], 36–45 days) following ICU admission. Patients were enrolled into the Coronavirus Outbreak Samples in New South Wales (COSIN) study and provided informed consent.[3]

Infection with the Delta variant was confirmed in all patients by determining the complete genome of isolated SARS-CoV-2 as previously described.[4] SARS-CoV-2 viral load dynamics were measured using real-time quantitative polymerase chain reaction (qPCR) on serial respiratory samples. All patients had at least one upper and lower respiratory paired sample tested with no detectable difference in viral load between sampling sites. As previously observed,[3,5] viral load decreased (increasing C_t, Figure 1A) with increasing time after disease onset. However, high viral loads ($C_t \leq 30$) persisted in all patients for median 35 days (IQR, 30–47 days) from disease onset. All patients had upper and lower respiratory samples collected between 10 and 35 days after disease onset, tested for viral culture using Hek293T cells expressing ACE2 receptor and TMPRSS2 serine protease (HekAT).[6] P1–P5 samples were culture negative at all timepoints examined, whereas P6 was culture-positive on Days 23 and 33 postdisease onset (Figure 1A).

Humoral antibody responses and Fc-mediated effector functions were analyzed in vitro on patient serum (Figure 1B). In patients P1–P5, neutralizing antibodies were measured (Chorus competition EIA) at median 1101 binding antibody units (BAU)/ml (IQR, 1085–1451 BAU/ml), indicative of high neutralizing capacity. In contrast, neutralizing antibody levels in P6 were below detection threshold at Day 0 and 3 after disease onset, but became detectable at a low level of 127.5 BAU/ml on Day 23. These results were consistent with neutralizing antibody titers determined by SARS-CoV-2 microneutralization assays performed with Vero E6 cells as we previously published.[6] Fc-mediated antibody-dependent cellular phagocytosis (ADCP) was investigated as Fc-mediated effector functions have been shown important for the establishment of protection and clearance of pathogens including SARS-CoV-2.[7] Using SARS-CoV-2 Spike protein-coated microbeads opsonized with antibodies from patient sera, all patients exhibited a detectable phagocytic response in at least one of the timepoints tested (Figure 1B). Compared to native patient sera, heat inactivation enhanced ADCP levels for P1–P5, suggesting the uncoupling of circulating antibody-virus complexes upon heat treatment. In contrast, heat inactivation did not enhance ADCP levels mediated by P6 sera in vitro. This is consistent with the lack of neutralizing antibodies and culture positivity observed for P6, further supporting the notion that a greater presence of "free" virions unbound by immune complexes contribute to higher and prolonged infectivity.

In Australia, as elsewhere, patients can be COVID-19 cleared and stepped down to non-COVID care pathways, provided individuals have complete resolution of symptoms and have been in isolation for

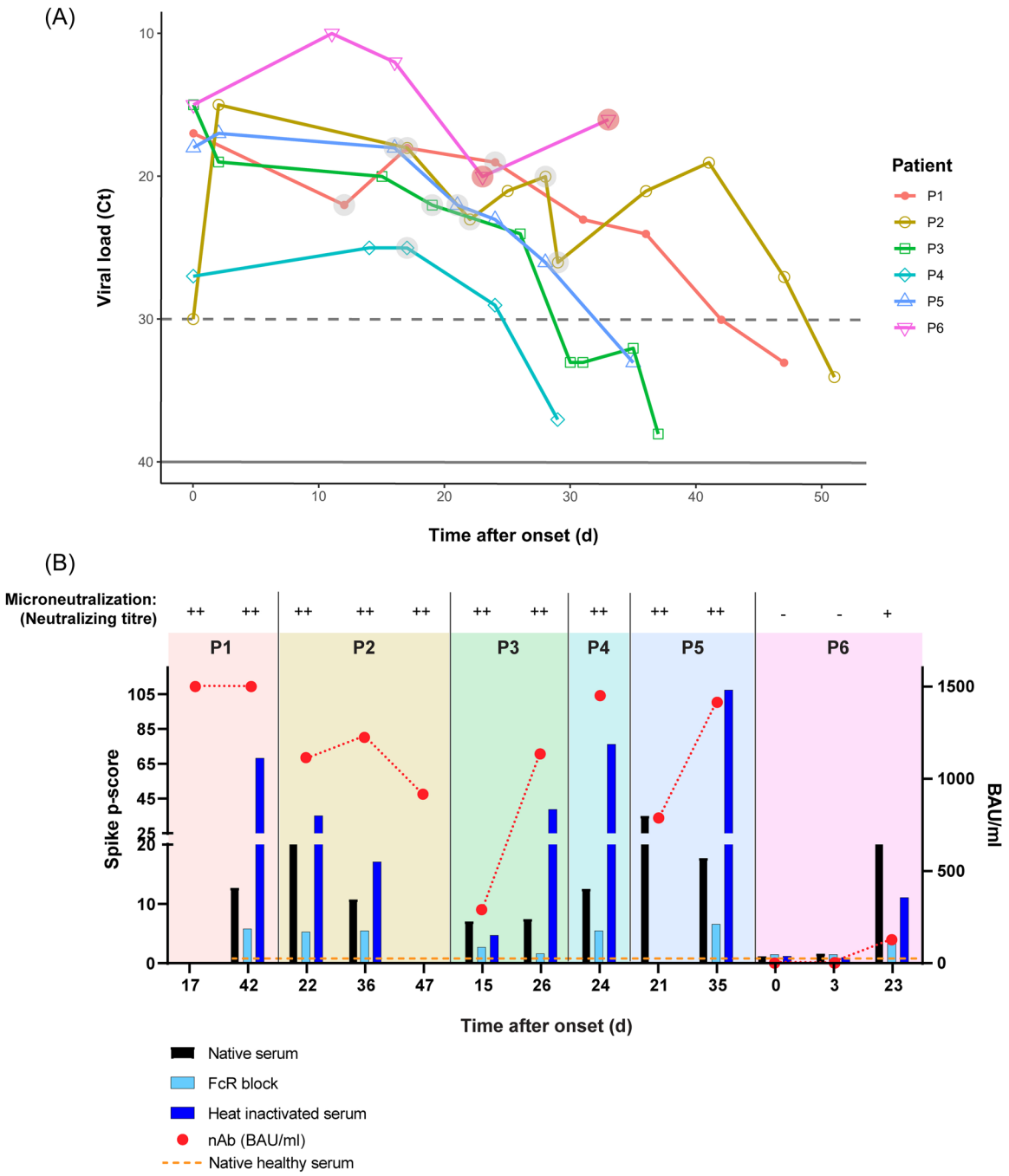

FIGURE 1 Persistent high-level shedding of SARS-CoV-2 Delta virus in COVID-19 patients admitted to the intensive care unit (ICU) with pneumonia. (A) Dynamics of the SARS-CoV-2 viral load by time after disease onset (D), measured using quantitative polymerase chain reaction targeting genomic regions E, N, and R of serial upper (nasopharyngeal swabs) and lower (bronchoalveolar lavage) respiratory tract specimens. These were taken from six patients (P1–P6) with severe COVID-19 during ICU admission. Higher cycle threshold (C_t) values correspond to lower viral load. C_t values did not differ between the two sample types. Colored bubbles indicate samples undergoing viral culture, with culture-negative (grey circles) and culture-positive (red circles) shown. Horizontal lines indicate C_t values 30 (dashed) and 40 (solid). (B) Antibody-dependent cellular phagocytosis (ADCP) by THP-1 cells of Spike protein-coated microbeads opsonized with patient sera showed detectable phagocytic response in all patients (black bars) defined as more than mean plus three standard deviations (SD) of phagocytic scores (*p* score). This score was obtained using sera from healthy donor controls ($n = 5$) as the baseline comparator (horizontal dashed line). Preblocking of the Fc-receptors on THP-1 cells abrogated the ADCP by 49%–78% (light blue bars), while heat inactivation of the patient sera caused a significant 0.6–6.3-fold enhancement of ADCP (blue bars) in patients P1–P5. Neutralizing antibody (nAb) responses (BAU/ml) measured at each timepoint using Chorus SARS-CoV-2 competition enzyme-immunoassay (EIA) are plotted in red on the secondary *y*-axis (right). These levels correlated with SARS-CoV-2 neutralizing antibody titers determined by microneutralization assays indicated at the top, expressed as high (++), low (+), or negative (−). Neutralizing antibody titers ≥40 were considered positive and high if ≥80. Microneutralization assays were performed using Vero E6 cells (2×10^4 cells per well).

minimum 14 days postsymptom onset.[8] These criteria are modified in the setting of immunosuppression or severe critical illness, with duration extended to at least 20 days and the addition of two negative PCR tests on consecutive respiratory specimens collected at least 24 h apart. The rationale for these latter requirements includes the delayed viral clearance in immunosuppressed patients, and ongoing infectivity demonstrated by culture positivity.[9–11]

Previous viral kinetic studies of patients infected with non-Delta variants admitted to the ICU show viral loads declining below culturable levels within 14 days.[12] In patients P1–P5 infected with the Delta variant, viral cultures were negative despite high viral loads (some with $C_t < 20$). In contrast, prolonged high-level virus shedding was accompanied by culture positivity up to 33 days after disease onset for patient P6. Given that these patients with severe COVID-19 due to the Delta strain required ICU support for a median of 43 days (IQR, 36–45 days), significantly longer than previously reported for non-Delta infections (median, 16 days; IQR, 9–28 days),[5] the ongoing high-level viral shedding brings uncertainties about COVID clearance and additional complexities to care. Of note, one feature (and accessible to most hospitals) that may help differentiate between patients who remain infectious to those that are not, are neutralizing antibody responses (Figure 1B).

Collectively, these data show that in the setting of severe COVID-19, patients infected with the Delta variant can shed high levels of infectious virus (determined by virus culture) and have low levels of neutralizing antibody (determined using competitive EIA and microneutralization) for prolonged times following presentation. These parameters should be monitored to inform infectivity and safe discharge from COVID care pathways, where relevant. Viral load determined using qPCR is an inadequate marker of infectivity and risk of onward transmission in this setting, where infection is with the Delta variant. Whether this holds for other emerging strains needs to be monitored prospectively.

Further investigation is required to determine whether these features of prolonged and persistent high-level viral shedding, are a consequence of infection with the Delta variant or are patient-specific. The increasing spread of the Delta, Omicron, and other emerging SARS-CoV-2 variants globally will present further complexities to COVID-19 management, especially for severe infections. Detailed laboratory assessment of viral load, infectivity, and immune response in selected patients with severe COVID-19 should begin early. Where appropriate, such assessment needs to include multiple measures of virus and host responses and should include laboratory review before discharge, to prevent unexpected onward transmission.

AUTHOR CONTRIBUTIONS

Ki W. Kim, Sebastiaan van Hal, William D. Rawlinson, and Rowena A. Bull designed the study and experiments. All authors formulated ideas, contributed to the interpretation of results, and reviewed the manuscript. Ki W. Kim, Malinna Yeang, Zin Naing, Frances Jenkins, and Anurag Adhikari performed the experiments. Ki W. Kim, Sebastiaan van Hal, and William D. Rawlinson wrote the manuscript.

ACKNOWLEDGEMENTS

The authors thank the participants of COSIN study and their families. Ki W. Kim was supported by a JDRF Postdoctoral Research Fellowship (3-PDF-2020-940-A-N). COSIN was supported by the Snow Medical Foundation, and WGS studies were supported by the Medical Research Futures Fund (MRFF).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The consensus SARS-CoV-2 genome sequence for the culture-positive case is available on GISAID (https://www.gisaid.org/; GISAID Accession: EPI_ISL_3874692). All other data supporting the findings of this case report are available upon reasonable request to the corresponding author (K.W.K.). The data are not publicly available due to privacy or ethical restrictions.

ETHICAL APPROVAL

Patient care and research were conducted in compliance with the Declaration of Helsinki. Experiments were performed with approval from the Human Research Ethics Committees of the Northern Sydney Local Health District and University of New South Wales, Australia (ETH00520). Patients were enrolled into the Coronavirus Outbreak Samples in New South Wales (COSIN) study and provided informed consent.

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