Duration of Infectious Virus Shedding in Patients With Severe Coronavirus Disease 2019 Who Required Mechanical Ventilation

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Abstract

Approximately 5% of patients with coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 develop severe COVID-19. Severe COVID-19 requires respiratory management with mechanical ventilation and an extended period of treatment. Prolonged infectious virus shedding is a concern in severe COVID-19 cases, but few reports have examined the duration of infectious virus shedding. Therefore, we investigated the duration of infectious virus shedding in patients transferred to Hiroshima University Hospital with severe COVID-19 requiring mechanical ventilation.

Nasopharyngeal swab specimens were collected and analyzed using both viral culture and reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) tests between December 2020 and February 2021.

Of the 23 patients tested, the proportions of those with positive test results at first specimen collection on RT-qPCR and viral culture tests were 95.7% and 30.4%, respectively. All six patients with positive viral culture test results who were followed-up tested negative 24 days after symptom onset but remained positive on RT-qPCR. The longest negative conversion time was observed in a dialysis patient on immunosuppressive drugs.

This study indicated that patients with severe COVID-19 remain culture positive for ≥ 10 days after symptom onset. Additionally, immunosuppressed patients with severe COVID-19 could consider isolation for ≥ 20 days.

Introduction

Cases of coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continue to increase worldwide. Approximately 5% of patients with COVID-19 develop severe COVID-19 and require mechanical ventilation¹. Numerous reports have shown that viral culture test results become negative 10 days after symptom onset^{2–4}. In mild cases, from day 10 onwards, isolation is recommended for 24 h after the disappearance of symptoms such as fever. In contrast, severe cases are believed to exhibit prolonged infectious virus shedding, and isolation for about 20 days is recommended by the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control^{5,6}. Nevertheless, an insufficient number of reports have examined the duration of infectious virus shedding in severe cases, and determining when to end isolation remains a major concern for medical institutions treating patients with severe COVID-19. A positive virus culture test result has been reported from a specimen collected 20 days after symptom onset⁷. A positive viral culture test result has even been reported on around day 70 after symptom onset in a patient with T-cell deficiency⁸.

We assessed the duration of virus shedding in patients with severe COVID-19 by performing viral culture and reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) testing of patients with

severe COVID-19 who required mechanical ventilation and were admitted to Hiroshima University Hospital for treatment.

Methods

Study participants and procedure

The study included 23 patients with severe COVID-19 who required mechanical ventilation and were admitted to Hiroshima University Hospital for treatment between December 2, 2020 and February 5, 2021. Hiroshima University Hospital accepts hospital transfers of patients with severe COVID-19 who require intubation management in Hiroshima Prefecture and provides treatment in its intensive care unit.

Nasopharyngeal swab specimens were collected with a swab and 1 mL of physiological saline. The first specimen was collected within one to two days after transfer to Hiroshima University Hospital. When the first viral culture test result was positive and circumstances allowed for follow-up, the duration of infectious virus shedding was investigated by collecting specimens every three to four days until negative viral culture conversion was observed. Patient information (date of symptom onset, medical history, medications, body mass index [BMI]) was extracted from the hospital's electronic medical records. Medical history of patients was only included in this study when there was evidence of a link between having a medical history and the occurrence of severe COVID-19⁹.

Cell and viral culture

SARS-CoV-2 was cultured in Vero cells expressing TMRPSS2 (VeroE6/TMRPSS2 cells, procured from JCRB cell bank, Japan). Cultures were observed for seven days and checked for cytopathic effects (CPEs). Infectivity titer was measured based on 50% tissue culture infectious dose (TCID₅₀), while TCID₅₀/mL was calculated using a method described in a previous report¹⁰. When no CPEs were observed within seven days, the culture was considered negative.

RT-qPCR

RT-qPCR tests were performed using a method described in a previous report¹⁰. SARS-CoV-2 nucleic acids were extracted using a Viral Total Nucleic Acid Purification Kit (Promega Corporation, USA), while RT-qPCR was performed using a One Step PrimeScript™ III RT-qPCR mix (Takara Bio Inc., Japan). N genes were targeted using the forward primer (2.4 μM), 5'-AAA TTT TGG GGA CCA GGA AC-3', reverse primer (3.2 μM), 5'-TGG CAG CTG TGT AGG TCA AC-3', and probe (0.4 μM) 5'-FAM-ATG TCG CGC ATT GGC ATG GA-BHQ-3'. RT-qPCR was run according to the manufacturer's protocol. A known quantity of SARS-CoV-2 RNA was used as a positive control, and copies/mL were calculated from cycle threshold (Ct) values.

Ethics

All methods were performed in accordance with the relevant guidelines and regulations. This research was approved by the Ethical Committee for Epidemiology of Hiroshima University (approval number: E-2157). The requirement for written informed consent was waived because only the patients' clinical specimens were used in this study, and the patients had the right to opt-out from the use of their surplus patient material and medical data for research.

Statistical analysis

Fisher's exact test and the Mann Whitney U test were used to compare patient characteristics. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using JMP software version 15 (SAS Institute Inc., USA).

Results

Patient characteristics

Thirty-three nasopharyngeal swab specimens were collected from 23 patients. Characteristics of patients included in the study are shown in Table 1. Patients were categorized into culture-positive (seven patients, 30.4%) and culture-negative (16 patients, 69.6%) groups, depending on initial viral culture test results. The median number of days prior to the first specimen collection was 10 days after symptom onset (culture-positive group: nine days, culture-negative group: 10 days; $p = 0.2358$). The median patient age was 64.0 years (culture-positive group: 67.0 years, culture-negative group: 62.5 years; $p = 0.6637$), and 82.6% of the patients were men (culture-positive group: 66.7%, culture-negative group: 87.5%; $p = 0.3518$). The median BMI was 28.4 kg/m^2 (culture-positive group: 26.4 kg/m^2 , culture-negative group: 30.8 kg/m^2 ; $p = 0.2291$), the most frequent comorbidity was hypertension (culture-positive group: 57.1%, culture-negative group: 50.0%; $p = 0.5555$), and 30.4% of patients had diabetes mellitus (culture-positive group: 14.3%, culture-negative group: 37.5%; $p = 0.2753$). No significant difference between the two groups was found in any characteristic.

Table 1
Patient Characteristics

Patient No.	Age (years)	Sex	First specimen collection (days after symptom onset)	Negative culture test (days after symptom onset)	BMI (kg/m ²)	Medical history
Culture-positive group (N = 7)						
P3	61	Male	12	24	20.3	Rheumatoid arthritis (receiving prednisolone and abatacept), end-stage renal failure (undergoing dialysis), autoimmune leukopenia
P7	48	Male	9	16	28.4	Fatty liver
P8	52	Male	10	N.D	32.4	Diabetes mellitus HbA1C: 11.2
P11	70	Male	9	13	26.4	Hypertension
P12	67	Male	10	13	25.7	Hypertension
P14	73	Female	8	15	24.5	Hypertension
P22	68	Female	4	11	27.9	Rheumatoid arthritis (receiving Janus kinase inhibitor and prednisolone), hypertension
Culture-negative group (N = 16)						
P1	53	Male	14	14	23.8	Diabetes mellitus HbA1C: 7.6, interstitial pneumonitis
P2	42	Male	11	11	34.7	-
P4	50	Male	11	11	40.7	Diabetes mellitus HbA1C: 9.8
P5	66	Male	9	9	31.0	Diabetes mellitus HbA1C: 8.7
P6	72	Male	10	10	29.7	Diabetes mellitus HbA1C: 11.9, hypertension
P9	68	Male	9	9	30.9	Diabetes mellitus HbA1C: 7.2, hypertension
P10	65	Male	8	8	26.1	Chronic hepatitis C

BMI, body mass index; HbA1C, glycated hemoglobin; P, patient; N.D, no data

Patient No.	Age (years)	Sex	First specimen collection (days after symptom onset)	Negative culture test (days after symptom onset)	BMI (kg/m ²)	Medical history
P13	57	Male	12	12	23.5	Hypertension
P15	81	Male	12	12	24.6	Hypertension
P16	77	Female	9	9	33.2	Diabetes mellitus HbA1C: 7.4
P17	68	Male	10	10	31.7	Hypertension
P18	64	Male	12	12	22.3	Emphysema, hypertension
P19	59	Male	6	6	23.7	Hypertension
P20	54	Male	10	10	30.7	Hypertension
P21	29	Male	13	13	36.4	Crohn's disease
P23	61	Female	8	8	32.6	Rheumatoid arthritis (receiving methotrexate)
BMI, body mass index; HbA1C, glycated hemoglobin; P, patient; N.D, no data						

Test results by specimen

At first specimen collection, 95.7% of the 23 patients tested positive via RT-qPCR results, and 30.4% of the patients tested positive via viral culture. Of the 33 specimens tested, the proportion of those with positive viral culture test results was 33.3%. The longest time to produce a positive viral culture test result was 21 days after symptom onset, and the infectivity titer ranged from 1.1×10^2 to 3.6×10^6 TCID₅₀/mL. Of the 33 specimens considered, the proportion of those with a positive RT-qPCR test result was 97.0% and detected viral loads ranged from 1.3×10^3 to 2.1×10^9 copies/mL (Fig. 1). RT-qPCR test results showed no change in viral load over time, but viral culture test results showed a decrease in infectivity titer over time.

Of the seven patients with an initial positive viral culture test result, specimens were collected and viral culture and RT-qPCR testing were performed in six patients until negative viral culture conversion occurred (Fig. 2). Viral culture testing revealed that the infectivity titer decreased over time and negative viral culture conversion occurred in all six patients (median negative conversion time: 14 days). PCR testing revealed viral loads of at least 10^4 copies/mL, even in specimens with a negative viral culture test result. One of the seven patients, whose negative viral culture conversion time was 24 days, had been receiving prednisolone and abatacept for rheumatoid arthritis, undergoing dialysis, and had underlying autoimmune leukopenia.

Discussion

In patients with severe COVID-19 requiring mechanical ventilation, a large difference in the percentage of positive results between viral culture and PCR testing was revealed. Viral culture testing revealed that viral load decreased over time, while decreases were not observed via PCR testing. Previous reports have also shown that PCR test results remain positive for an extended period, up to 40 days or more in some cases^{11,12}. The CDC does not recommend ending isolation based on PCR testing⁵. The results of this study also showed that it is difficult to evaluate whether a patient is infectious based on PCR testing, even if the viral load is known.

In terms of infectivity titers, we confirmed that both the viral load and percentage of positive results decreased over time. One patient required 24 days for negative viral culture conversion to occur. In this patient, factors associated with immunosuppression, including treatment with prednisolone and abatacept, and dialysis for renal failure likely influenced the results. The likelihood that these factors caused severe COVID-19 has already been identified. Previous studies revealed that a high proportion of patients receiving prednisolone for rheumatism developed severe COVID-19¹³, and the mortality rate of COVID-19 is reportedly higher in patients who are on dialysis than in their counterparts¹⁴. Nevertheless, the possibility that these factors may lead to viral culture test positivity for prolonged periods has not been fully examined. Though this affected only one patient in this study, the finding suggests that these factors may prolong the negative viral culture conversion time.

For all the other patients in this study, there was no difference in age, BMI, or medical history between the culture-positive and culture-negative groups, and negative viral culture conversion occurred between days 6 and 16 after symptom onset. Furthermore, in this study, approximately one third (30·4%) of all patients with severe COVID-19 were believed to have been shedding the infectious virus 10 days after symptom onset.

This study has a few limitations. This was a single-center study of a group with a small sample size, and only seven patients had positive viral culture test results. Patients were transferred to our hospital when they required mechanical ventilation and the timing of the first specimen collection was different for each patient. A few specimens were collected within seven days after symptom onset, and we could not investigate the percentage of positive viral culture test results obtained prior to initiating therapy. SARS-CoV-2 infectivity was assessed with a viral culture test that used Vero cells expressing TMPRSS2. Vero cells are well suited for SARS-CoV-2 culture¹⁵, and Vero cells expressing TMPRSS2 are even more susceptible to SARS-CoV-2 infection¹⁶. However, additional knowledge is required to investigate what factors inhibit viral culture and the detection limits of viral culture.

This study showed that 30% of the critically-ill patients remained culture positive for ≥ 10 days after symptom onset. Further, one immunosuppressed patient required 24 days of testing before negative viral culture conversion occurred, suggesting that a longer period of isolation is required for patients with

impaired cellular immunity. Further research investigating patients with COVID-19 who exhibit prolonged infectious virus shedding is required.

Declarations

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None.

Author Contributors

This study was designed and supervised by NS, TS, and HO. T. Nomura, HK, KO, and NS collected samples and clinical data, which were analyzed by T. Nomura, MK, and TN. All authors contributed to interpreting the data. The final manuscript was reviewed by all authors.

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Competing interests

We declare no competing interests.

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Figures

Figure 1

Quantitative results by type of test (N = 33) PCR, polymerase chain reaction

Figure 2

Time-course of conversion in patients with positive culture test and in patients with positive RT-qPCR test
RT-qPCR, reverse transcriptase-quantitative polymerase chain reaction