

Title

A patient infected with SARS-CoV-2 over 100 days

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Learning Point for Clinicians

We recently experienced a patient with viral isolation by culture 4 months after the acute

infection. COVID-19 has manifested in many unexpected forms throughout its short existence.

Patients with ongoing symptoms of COVID-19, long-haulers, could be chronically infected with

COVID-19, although they might be incapable of transmission.

On February 28th, 2020, shortly before the outbreak of the COVID-19 pandemic in our local area, an 83-year-old Japanese man visited our outpatient clinic in Tsukuba Memorial Hospital with symptoms of upper respiratory tract infection. His medical history included asthma, lung operation due to cryptococcus, and mild dementia. His clinical course is shown in Figure 1.

Twelve days later, after his symptoms did not abate, he was admitted to our hospital, but laboratory tests and a computed tomography (CT) revealed no significant findings. He subsequently developed a fever and hypoxia. X-ray showed an infiltrative shadow, although he was administered several antibiotics including levofloxacin and methylprednisolone. Bilateral ground-glass opacification and consolidation were revealed in a second CT.

One month after his initial clinic visit, the patient was diagnosed with a COVID-19 infection by a quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) test. His PaO₂/FiO₂ fell to 160, and he became unconscious. The trachea was intubated, and he was transferred to ICU at Tsukuba University Hospital, where he was given a tracheostomy and administered favipiravir, antibiotics, antifungals, and edoxaban. Two months after his initial clinic visit, he was returned to the ICU at Tsukuba Memorial Hospital; his tracheostomy was closed after 3 months, and his

quarantine for infection control was discontinued. By this time, he had regained most of his pre-COVID-19 level of health, although there was muscle weakness and progression of his dementia.

However, qRT-PCR tests continued to be positive. At day 111, specimens from a nasopharyngeal swab and sputum were inoculated into VeroE6/TMPRSS2 cells.(1) Three to five days post-inoculation, cytopathic effects were observed, and viral RNA was detected in the culture supernatant by qRT-PCR (Ct values: 29–37). The culture supernatant of the VeroE6/TMPRSS2 cells cocultured with the nasopharyngeal swab was passaged to the fresh VeroE6/TMPRSS2 cells, and after 3 days of the passage, cytopathic effect was observed again. In the supernatant of the passaged cell culture, viral RNA was detected by qRT-PCR (Ct value: 29). By sequencing the qRT-PCR product using Illumina iSEQ100, the presence of SARS-CoV-2 RNA sequence was verified. These results indicate the presence of infectious SARS-CoV-2 in the nasopharyngeal specimen even at day 111. Although SARS-CoV-2 was present, the patient developed neutralizing antibodies by a microtiter method.(2) We tested but did not isolate SARS-CoV-2 in

samples of saliva, urine, blood, or stool; no health care personnel who were in contact with the patient became infected with COVID-19 after the patient left quarantine.

COVID-19 patients with tracheostomies were usually kept in quarantine, especially when qRT-PCR tests remain positive. Criteria for hospital discharge or discontinuation of quarantine, however, have changed as new clinical features of COVID-19 have emerged, and negative qRT-PCR tests are no longer necessary.(3)

Our patient might be like other individuals (now known as “long-haulers”), who have reported chronic, ongoing symptoms of COVID-19 weeks or months after the initial diagnosis, but there have been no reported cases where SARS-CoV-2 continued to be isolated by viral culture. Some previous studies about COVID-19 suggest that recovery can be achieved only by completely clearing the virus from the body.(3, 4) However, COVID-19 has manifested in many unexpected forms throughout its short existence: infecting some individuals but leaving them asymptomatic, exacting a heavy death toll on the elderly population, and leaving some individuals, “the long-haulers,” with debilitating, long-term symptoms.

Our patient continued to test positive 4 months after the initial infection and this case may indicate yet another disease trajectory for COVID-19 infections. Some patients with ongoing symptoms of COVID-19,(5) long-termers or long-haulers,(6) could be chronically infected with COVID-19, although they might be incapable of transmission.

Declarations

Conflict of interest: None

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Author contributions

TA identified a case described and was a major contributor in writing the manuscript. TI, JI, YS, CN, and KS contributed viral isolation. TI, YT, and KS assisted in the manuscript revision. All authors read and approved the final manuscript.

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Figure Legend

Figure 1 Clinical course of the patient

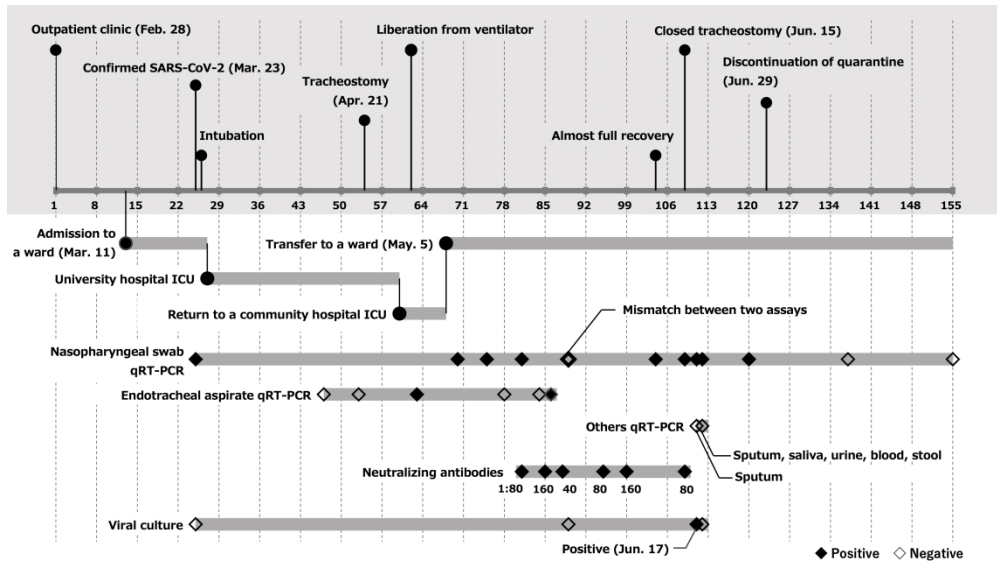


Figure 1 Clinical course of the patient

338x190mm (300 x 300 DPI)

Abbreviation List

quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR); computed tomography (CT);

Abstract

We recently experienced a patient with viral isolation by culture 4 months after the acute infection. An 83-year-old Japanese man visited our outpatient clinic with symptoms of upper respiratory tract infection. The patient was diagnosed with a COVID-19 infection by a quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) test. Three months after his clinic visit, he had regained most of his pre-COVID-19 level of health.

However, qRT-PCR tests continued to be positive. At day 111, specimens from a nasopharyngeal swab and sputum were inoculated into VeroE6/TMPRSS2 cells.⁽¹⁾ Three to five days post-inoculation, cytopathic effects were observed, and viral RNA was detected in the culture supernatant by qRT-PCR (Ct values: 29–37). The culture supernatant of the VeroE6/TMPRSS2 cells cocultured with the nasopharyngeal swab was passaged to the fresh VeroE6/TMPRSS2 cells, and after 3 days of the passage, cytopathic effect was observed again. In the supernatant of the passaged cell culture, viral RNA was detected by qRT-PCR (Ct value: 29). By sequencing the qRT-PCR product using Illumina iSEQ100, the presence of SARS-CoV-2 RNA sequence was verified. These results indicate the presence of infectious SARS-CoV-2 in the nasopharyngeal specimen even at day 111. Although SARS-CoV-2 was present, the patient developed

neutralizing antibodies by a microtiter method. We tested but did not isolate SARS-CoV-2 in samples of saliva, urine, blood, or stool.

Patients with ongoing symptoms of COVID-19, long-haulers, could be chronically infected with COVID-19, although they might be incapable of transmission.