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Running head: Long-term detection of SARS-CoV-2

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**ABSTRACT:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019, is spreading globally. In general, the viral genome becomes undetectable within a couple of weeks after infection. Herein, we report a case of long-term detection of the SARS-CoV-2 genome from the same individual for 106 days. Whole genome sequencing was performed on specimens taken at the onset of the disease and at 2 months after onset, and the B.1.1.7 lineage was detected in both samples. Comparison of the full-length sequences revealed a single-base difference and no amino acid mutations. This is the first case in Japan where the virus was detected over a long time, and the full-length sequences were compared.
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the genus *Betacoronavirus* in the *Coronaviridae* family. The SARS-CoV-2 genome is a 29.9 kb positive-sense single-stranded RNA, whose size is the largest among RNA viruses (1). In Japan, PCR testing is used for the detection of the virus from clinical specimens, such as pharyngeal and nasopharyngeal swabs, saliva, and sputum (2). The virus detection period by PCR testing is approximately 14.5 days in the case of upper respiratory tract specimens (3); however, in some cases, the viral genome has been detected for more than a month after the onset of illness (4, 5, 6).

Next-generation sequencing (NGS) is useful not only for the detection of SARS-CoV-2 but also for the phylogenetic classification of the virus. Since NGS allows detailed genomic analysis at the single nucleotide level, the analysis of the whole genome of SARS-CoV-2 can reveal the different viral clusters and reinfection episodes (7, 8, 9).

Here, we report a case in which the SARS-CoV-2 genome was detected in a hospitalized patient for as long as 106 days after the onset of illness. In addition, we show the whole viral genome sequences obtained from this patient immediately after disease onset and 2 months later.

A 79-year-old woman was admitted to a medical institution for cerebral hemorrhage on April 27, 2021. She presented with a fever (38.1 °C) on May 30, 2021, which was defined as day 0 of disease onset. Although the symptoms subsided later, she had vomiting, and SARS-CoV-2 PCR testing was positive on June 4 (5 days after disease onset). The patient had been in contact with a SARS-CoV-2-infected person before May 30, which may have been the time that she became infected; there was no apparent history of contact with any other infected person. Notably, she was not vaccinated against coronavirus disease 2019 (COVID-19) and had underlying medical conditions, including cerebral hemorrhage, hypertension, and dyslipidemia. Prior to the hospitalization for cerebral hemorrhage, the patient was receiving atorvastatin, telmisartan, and
suvorexant from a primary care clinic. After admission, she received continuous intravenous nicardipine (4 days) for hypertension. The day after admission, the patient received haloperidol infusions twice for delirium. On days 6 and 27 after disease onset, she had a fever of over 37.5 °C and an occasional cough (Fig. 1). On day 70 after onset, vomiting occurred. Oxygen was administered from June 4 to 22 to manage the moderately severe COVID-19. The patient also received heparin from June 11 to July 2 to prevent thrombus.

Nasopharyngeal swab samples were collected 20 times (on June 4, 17, and 24; July 1, 12, 19, 26, and 30; August 2, 5, 6, 10, 16, 26, 30, and 31; and September 6, 13, 21, and 22) from the patient and subjected to PCR testing using the SARS-CoV-2 Direct Detection RT-qPCR Kit (Takara Bio Inc., Kusatsu, Japan). Viral loads were assessed using the Ct values obtained by PCR testing. All specimens were positive except for those collected on July 30, August 30, and September 21 and 22. The patient showed a low Ct value after disease onset, but the Ct value increased after 43 days. At 68 days after COVID-19 onset, the Ct value was 26, and the viral genome was detectable until 106 days after disease onset (September 13).

Whole genome sequencing was performed on two nasopharyngeal swab samples collected on June 4 and August 6, 2021. The analysis method was based on the method reported by Itokawa et al. (10, 11). The obtained gene sequences were registered in the GISAID database (EPI_ISL_3183025 and EPI_ISL_5755738). The B.1.1.7 (alpha) lineage was detected in both viral samples. The difference between the two samples was a single nucleotide mutation (A6646G compared to the Wuhan-Hu-1, Genebank accession number: NC-045512), and no differences were observed in the amino acid sequence. In addition, on September 16 (109 days after disease onset), serum antibodies were measured using Elecsys Anti-SARS-CoV-2 (Roche, Basel, Switzerland) and Architect SARS-CoV-2 IgG (Abbott, Chicago, USA), and immunoglobulin against the virus
was detected. This implies that the capacity of the patient to generate antibodies has been maintained.

In this study, we encountered a case in which SARS-CoV-2 genes were detectable for a long period (106 days). The long-term viral detection could be due to virus gene mutations, SARS-CoV-2 reinfection, and host immune responses. However, whole genome analysis showed that there were no amino acid mutations; therefore, it is unlikely that the pathogenicity of the virus changed due to viral gene mutations. Since the patient was hospitalized and had no contact with SARS-CoV-2-positive individuals since the onset of the disease, SARS-CoV-2 reinfection was unlikely. Additionally, most of the B.1.1.7 SARS-CoV-2 isolates detected in Ibaraki, Japan, around August, were replaced by B.1.617.2 isolates; therefore, the possibility of reinfection by B.1.1.7 strains was low. Whole genome analysis ruled out reinfection with a different strain. Of note, the patient had no underlying immunodeficiency and was not taking immunosuppressive drugs. The treatment administered to this patient during the virus detection period was oxygen and heparin for thromboprophylaxis. There was no decrease in white blood cells or lymphocytes, and antibodies against SARS-CoV-2 were detected. The mechanism of long-term detection of SARS-CoV-2 is unknown, but it is possible that old age, host’s genetic environment, other comorbidities, severity of the illness, nutritional status, and previous exposure to the virus may have led to long-term detection of the virus (12).

Long-term detection of the virus has typically been reported in immunodeficient individuals or in those receiving immunosuppressive treatment, in addition to detection because of genetic mutations of the virus (13). Such cases are often associated with multiple mutations in the viral genome. However, in the case reported here, the patient was not immunosuppressed, and the genetic changes during the 2-month period were synonymous substitutions of single
nucleotides. Nakagawa et al. reported that there are 26 substitutions (on average) in the SARS-CoV-2 genome per year (14). In this case, the number of genetic mutations was very small, considering the time when the virus was detected. To the best of our knowledge, this is the first case report of a Japanese patient with persistent detection of the virus for 106 days.

Acknowledgments

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Conflict of interest

None to declare.

References


Figure legends

Fig. 1. PCR results and the patient’s clinical course. Fever is listed as 37.5 °C or higher. The duration of the cough is presented in black. The gray area represents occasional coughing. Ct values were determined using the SARS-CoV-2 direct detection RT-qPCR kit (Takara).
Days after symptom onset

PCR

Ct value

Whole genome sequence

Symptom

Fever 38.1 38.4 37.9

Vomiting

Cough

Oxygen

Heparin

Medication

0 10 20 30 40 50 60 70 80 90 100 110