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SHORT COMMUNICATION

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Sequential dynamics of virological and serological changes in the serum of SARS-CoV-2 infected patients

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load dynamics in respiratory samples have been studied, but knowledge about changes in serial serum samples of infected patients in relation to their immunological response is lacking. We investigated the dynamics of SARS-CoV-2 viral load and antibody response in sequential serum of coronavirus disease 2019 (COVID-19) patients and attempted to culture the virus in the serum. A total of 81 sequential serum samples from 10 confirmed COVID-19 patients (5 with mild and 5 with moderate symptoms) were analyzed. Samples were collected during hospitalization and after discharge (median follow-up of 35 days). SARS-CoV-2 ribonucleic acid in the serum was detected by real-time polymerase chain reaction. Total antibody and IgG to SARS-CoV-2 Spike protein were analyzed by Chemiluminescent Immunoassays, and neutralizing antibodies were detected using a Surrogate Virus Neutralization Test. Viremia was observed in all cases at admission, and viral copy gradually dropped to undetectable levels in patients with mild symptoms but fluctuated and remained persistent in moderate cases. The viral culture of samples with the highest viral load for each patient did not show any cytopathic change. The antibody response was faster and higher in moderate cases. This study provides a basic clue for infectious severity-dependent immune response, viremia, and antibody acquisition pattern.

KEYWORDS

antibody, COVID-19, SARS-CoV-2, serum, viral culture, viremia

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic remains a considerable health problem globally, with a cumulative 216 million people infected in the world as of August 30, 2021.[1] The identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time reverse-transcriptase polymerase chain reaction (RT-qPCR) in upper respiratory samples is the gold standard for diagnosis. However, SARS-CoV-2 can be identified in various specimens, including blood, urine, and feces.[2] SARS-CoV-2 viral load dynamics in respiratory samples have already been studied, but knowledge of the viral load pattern in serial serum samples of infected patients in relation to their immunological

response is lacking. Although previously published studies did not find evidence of infectivity of SARS-CoV-2 in the blood,[3,4] it is essential to assess the viremia status based on disease severity. Also, the antibody response following infection with SARS-CoV-2 is still under investigation, especially the acquisition of neutralizing antibodies.

Using sequential serum of COVID-19 patients, we aimed to investigate the dynamics of SARS-CoV-2 viral load and the antibody response after infection. Additionally, we attempted to culture the virus in the serum.

2 | MATERIALS AND METHODS

Between July 27 and December 17, 2020, ten confirmed COVID-19 patients by PCR testing of nasopharyngeal samples from the Hiroshima City Funairi Citizens Hospital were enrolled.

Clinical data were derived from medical records. The severity of the infection was based on the National Institutes of Health criteria.[5] A total of 81 whole blood samples were collected from patients at the time of their hospitalization and at different time points during and after discharge. The blood was centrifuged, and serum was stored at −80°C until laboratory analyses. Total antibodies to the SARS-CoV-2 Spike protein (IgG, IgM, and IgA) were detected by chemiluminescent immunoassay (CLEIA) using VITROS Anti-SARS-CoV-2 Total (Ortho Clinical Diagnostics). The VITROS Anti-SARS-CoV-2 IgG CLEIA (Ortho Clinical Diagnostics) was used to detect immunoglobulin G (IgG) to the SARS-CoV-2 spike protein. Neutralizing antibodies to SARS-CoV-2 were detected using the GenScript SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) (GenScript), an ELISA-based assay that mimics the viral neutralizing process. The sVNT was considered positive when the inhibition rate was ≥ 30%.

In addition to serological analyses, we investigated the presence of SARS-CoV-2 in the serum of patients. First, the viral ribonucleic acid (RNA) was extracted using the SMITEST EX-R&D kit (MBLA) and quantified by RT-qPCR (Thermo Fisher Scientific). Primers and probes developed by the Japan National Institute of Infectious Diseases and targeting the nucleocapsid gene were used, namely 2019-n-CoV-N-F2 (position nt29,125–nt29,144), NIID_2019-n-CoV-N-R2 (position nt29,299–nt29,280), and NIID_2019-n-CoV-N-P2 (position nt29,222–nt29,241). The full method of RNA quantification is described elsewhere.[6] Then, for each patient, the sample with the highest viral load was selected and cultured in Vero-E6 cells expressing human Transmembrane protease, serine 2 (TMPRSS2) after 5–20 fold dilution, using the same method as Kitagawa et al.[7] Experiments were performed in duplicate, and when no cytopathic change was observed after 5 days, the culture was considered negative.

This study was approved by the Ethics Committee of Hiroshima University (E-2124) and conducted according to the Helsinki declaration. Before any study procedures, all patients provided their informed consent.

3 | RESULTS

3.1 | Characteristics of the patients

A total of 10 patients were enrolled, including six men and four women. The age ranged from 25 to 76 years, with a median age of 60 years. Half of the patients had mild symptoms, and the other half had moderate symptoms. The hospitalization duration ranged from 9 to 25 days (median = 13 days), and the follow-up duration was between 20 and 66 days (median = 35 days). No death was observed during the follow-up period. Detailed characteristics of the patients are presented in Table 1.

3.2 | Total antibody, IgG, neutralizing antibodies, and viral load

Figure 1 shows the dynamics of Total antibody, IgG, neutralizing antibodies, and the viral load in the serum of all patients.

TABLE 1 Characteristics of COVID-19 patients

Patient	Age (years)	Sex	Smoking Status	Underlying disease	Severity of symptoms	Duration of hospitalization (days)	Follow-up duration (days)	Follow-up outcome
P01	56	Male	Never	Hypertension	Mild	11	66	Alive
P02	46	Female	Current	None	Mild	11	24	Alive
P03	25	Male	Never	None	Mild	9	22	Alive
P04	46	Male	Current	None	Mild	9	27	Alive
P05	67	Male	Never	Stroke	Mild	22	34	Alive
P06	76	Female	Never	Hypertension	Moderate	16	40	Alive
P07	63	Female	Never	Diabetes Mellitus	Moderate	20	41	Alive
P08	57	Male	Never	Diabetes Mellitus	Moderate	25	20	Alive
P09	74	Female	Past	Hypertension	Moderate	12	44	Alive
P10	63	Male	Past	None	Moderate	14	36	Alive

Abbreviation: COVID-19, coronavirus disease 2019.

SARS-CoV-2 RNA was detected in the serum of all patients at admission. In patients with mild symptoms, viral copy at admission ranged from 10.9 to 1299.1 copies/ml and became undetectable in 4/5 patients at the end of follow-up. The viral RNA remained detectable in one patient with mild symptoms (P01, Figure 1) with a viral load of 19.9 copies/ml. In patients with moderate symptoms, the viral load at admission ranged from 19.5 to 231.0 copies/ml and fluctuated throughout the follow-up. However, the viral RNA remained detectable at the end of the follow-up in all patients, ranging from 19.5 to 316.1 copies/ml.

The viral culture of the samples with the highest viral load for each patient showed no cytopathic change after five days.

In all patients, total antibody, IgG, and neutralizing antibodies started rising within a week after symptoms onset. In both mild and moderate cases, sVNT was positive within two weeks (inhibition rate≥ 30%) and continuously increased. However, the rise of the neutralizing antibodies was faster with a higher inhibition rate in patients with moderate symptoms than those with mild symptoms.

4 | DISCUSSION

This study assessed SARS-CoV-2 viral load and antibody response dynamics in sequential serum samples of COVID-19 patients.

We found that viremia was observed in the serum of COVID-19 patients from admission and viral copy decreased in mild cases to undetectable levels. However, it fluctuated throughout the observation period and was still detectable several days after symptoms onset in moderate cases. Our results suggest that a one-time assessment of viral load is not enough to determine viremia. From a clinical point of view, previous studies have reported that SARS-CoV-2 viremia is associated with disease severity and mortality.[8,9] In our study, no death was observed at the end of the follow-up.

Although SARS-CoV-2 was detected in the serum, the viral culture was negative. This finding is in line with previous reports.[3,4] To date, no study evidenced the infectivity of SARS-CoV-2 in blood samples, and there is no reported case of transmission via transfusion. The selection of samples with the highest viral load for each patient in our study provides additional evidence of the absence of infectivity in the serum of COVID-19 patients.

The antibody response was faster and higher in patients with moderate symptoms compared with those with mild symptoms. The same findings were reported by Imai et al. in Japanese patients.[10] The neutralizing antibodies were related to the IgG in both mild and moderate patients, but the inhibition rate was higher in moderate cases, suggesting that disease severity affects the antibody response.

Limitations of our study include the small sample size and variability in patient age, which may affect the pattern of the immune response and viremia clearance. In addition, the absence of paired upper respiratory samples to compare the viral load dynamics in the respiratory tract and serum, the difference in timing of blood sampling, and the duration of follow-up suggest a cautious interpretation

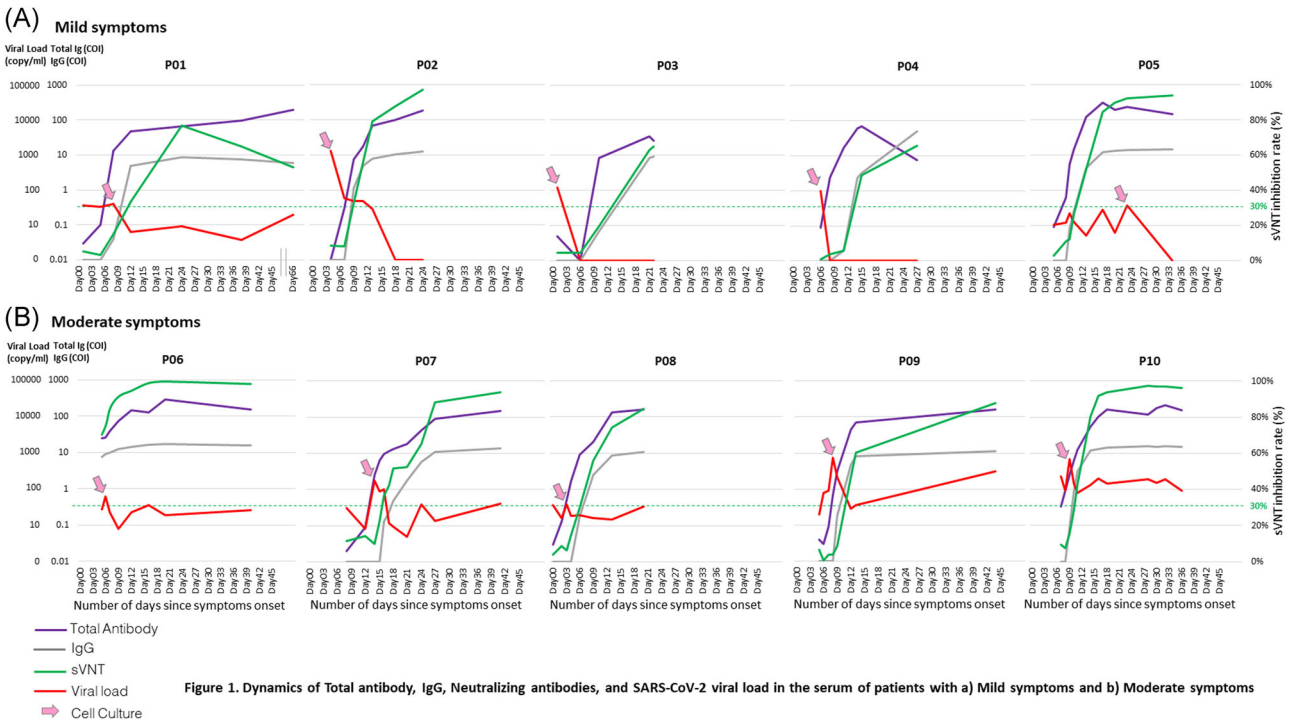

FIGURE 1 Dynamics of total antibody, IgG, Neutralizing antibodies, and SARS-CoV-2 viral load in the serum of patients with (A) Mild symptoms and (B) Moderate symptoms. The surrogate virus neutralization test (sVNT) for neutralizing antibodies was considered positive when the inhibition rate was ≥ 30%. The arrows show the samples selected for viral culture. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

of our results. Replication studies with a large sample size are needed to confirm our findings.

In conclusion, SARS-Cov-2 was detected in low levels in the serum of COVID-19 patients and remained detectable several days after symptoms onset in patients with moderate symptoms, but the serum was not infectious. Antibody response occurred within a week and was higher and faster in patients with moderate symptoms than those with mild symptoms. Thus, this study provides a basic clue for infectious severity-dependent immune response, viremia, and antibody acquisition pattern.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Serge Ouoba: Methodology, Formal analysis, Investigation, Writing - original draft. **Mafumi Okimoto**: Investigation. **Shintaro Nagashima**: Methodology, Formal analysis, Investigation, Writing - original draft. **Yoshihiro Kitahara**: Investigation. **Kei Miwata**: Investigation. **Ko Ko**: Methodology, Formal analysis, Investigation, Writing - original draft. **Bunthen E**: Methodology, Formal analysis, Investigation, Writing - original draft. **Aya Sugiyama**: Methodology, Investigation. **Kazuaki Takahashi**: Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing - review & editing. **Takemasa Sakaguchi**: Methodology, Validation, Writing - review & editing. **Toshiro Takafuta**: Conceptualization, Supervision. **Junko Tanaka**: Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Writing - review & editing.

DATA AVAILABILITY STATEMENT

All the data included in this study were fully described in the manuscript.

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