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Case Report

A Case of Persistent SARS-CoV-2 PCR Positive after Chemotherapy for B-cell Lymphoma

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Summary

The patient was a 68-year-old woman who was hospitalized for SARS-CoV-2 pneumonia while receiving chemotherapy for B-cell lymphoma. She experienced repeated episodes of pneumonia that temporarily went into remission with steroid and remdesivir and but then relapsed or worsened. However, no particular subjective symptoms or deterioration on imaging was observed by an antibody cocktail treatment after Day 119. SARS-CoV-2 IgM and IgG antibody levels were below the limit of detection on Day 180 but she tested positive for SARS-CoV-2 by PCR on Day 151. In the case of this patient who had persistent SARS-CoV-2 infection due to being immunocompromised, treatment temporarily stalled the worsening of symptoms. However, new treatments for such patients should be established soon.

Key Words: B-cell lymphoma, chemotherapy, SARS-CoV-2, pneumonia, antibody cocktail, immunocompromised host

Introduction

Generally, when a person is infected with severe acute respiratory syndrome coronavirus (SARS-CoV)-2, immunity is acquired and activation and proliferation of natural killer (NK) cells triggers destruction of viral cells[2)]. It is also believed that at the same time, the body produces neutralizing antibodies against the spike protein on the surface of SARS-CoV-2, and this response inhibits infection of uninfected cells[1)]. We present the case of a patient with long-term of SARS-CoV-2 PCR positive persistent after chemotherapy for B cell lymphoma.

Case Presentation

The case was a 68-year-old woman with a past medical history of diabetes (relatively well controlled with oral administration of sitagliptin phosphate hydrate 50 mg/day and glimepiride 1 mg/day), hypertension, and bronchial asthma received four cycles of bendamustine plus rituximab for about four months to treat the B-cell lymphoma (stage IV, mucosa associated lymphoid

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tissue lymphoma). Four days after the fourth cycle of bendamustine plus rituximab, she was found to have SARS-CoV-2 infection. The source of infection was a family member. She had not received a SARS-CoV-2 vaccine. Vital signs included blood pressure of 143/68 mmHg, heart rate of 92 beats/min, body temperature of 36.5 ℃, and SpO_2 of 98% (room air). Laboratory data were as follows: white blood cells (WBC) 700 cells/μL, neutrophils 76.0%, lymphocytes 7.0%, monocytes 16.0%, eosinophils 1.0%, red blood cells 2.64 × 10^6/uL, hemoglobin 9.0 g/dL, platelets 10.0 × 10^4/μL, D-dimer 0.5 μg/mL, lactate dehydrogenase 213 U/L, C-reactive protein 0.30 mg/dL, ferritin 266.25 ng/dL, HbA1c 6.5%, immunoglobulin (Ig) M 12 mg/dL, IgG 813 mg/dL (measured between 3 days before or 3 days after onset of illness), serum soluble interleukin 2 receptor 368 U/mL. SARS-CoV-2 pneumonia was diagnosed based on diffuse ground glass opacities detected in the right lower lobe on chest CT (Fig. 1a), and the patient was admitted to our hospital on the same day. The pneumonia was not severe enough to require oxygen therapy, and thus was judged to be moderate I severity according to the classification system of the Japanese Ministry of Health, Labour and Welfare[2]. Informed consent was obtained from the patient for publication of this case report.

On Day 14, polymerase chain reaction (PCR) testing for SARS-CoV-2 by collecting saliva showed the presence of the E484K mutation. Until Day 25, no decrease in SpO_2 on room air was observed and there was no change in symptoms, so the COVID-19 pneumonia was not treated and the course was carefully monitored. A chest X-ray (Fig. 1b) and chest CT scan (Fig. 1c) on Day 26 showed that the ground glass opacities in left and right lung fields had become more prominent, which was judged to indicate progression of SARS-CoV-2 pneumonia. Treatment with dexamethasone, dalteparin sodium, and remdesivir was started that day and continued until Day 35. During that time, the patient experienced moderate exacerbations of pneumonia, none of which were severe enough to warrant a transfer to the ICU. These repeated episodes, which were judged to be persistent pneumonia of moderate I to II severity according to the classification system[2], were treated successfully with dexamethasone and remdesivir, and are regarded as the first two exacerbations and remissions (Fig. 1d-f). Other treatments, including favipiravir and γ-globulin monotherapy, were also attempted but had no effect on the pneumonia. Considering that this patient was immunocompromised, was experiencing repeated episodes (4 exacerbations) of pneumonia due to persistent SARS-CoV-2 infection, and the levels of IgM and IgG antibodies against SARS-CoV-2 were below the detection limit, an antibody cocktail (casirivimab + imdevimab) approved for manufacturing and marketing but not covered by Japanese National Health insurance was administered on Day 119 with the permission of the patient, her family, and the extraordinary bioethics committee of our hospital for non-applicable use. No particular subjective symptoms or deterioration on imaging was observed, but considering the general half-life of the antibodies in the blood, a second antibody cocktail treatment was administered on Day 132 after another positive SARS-CoV-2 PCR result on the day. The SARS-CoV-2 PCR result on Day 138 was still positive, and the pneumonia had neither relapsed nor progressed after antibody cocktail therapy. Consequently, the patient was discharged on Day 139 and followed on an outpatient basis after a period of recovery at home. After discharge, the SARS-CoV-2 PCR by collecting saliva was still positive on Day 151, but was negative on Day 180, and her condition remained stable. These results suggested that the cocktail therapy could be considered effective for a control of the exacerbation to SARS-CoV-2 pneumonia.

The detailed clinical course is shown in Fig. 2. Various culture tests could not be performed because sputum production was difficult. Blood cultures were performed on Days 19 and 26 but the results were negative. On Days 19, 26, and 49, β-D glucan and aspergillus antigens were tested, but none of the results were positive or suspicious. However, fungal infection could not be completely ruled out during the second exacerbation, so antifungal drugs were administered. Bacterial infection could not be completely ruled out at the time of exacerbation, so antibacterial agents were used as appropriate. WBC ranged from about 600 to 6000 cells/μL, and the total IgG level remained low, ranging from about 400 to 500 mg/dL. The total $CD4^+$ T cell count was 15/μL on Day 82, 124/μL on Day 124, 223/μL on Day 151, and 192/μL on Day 180. The values of

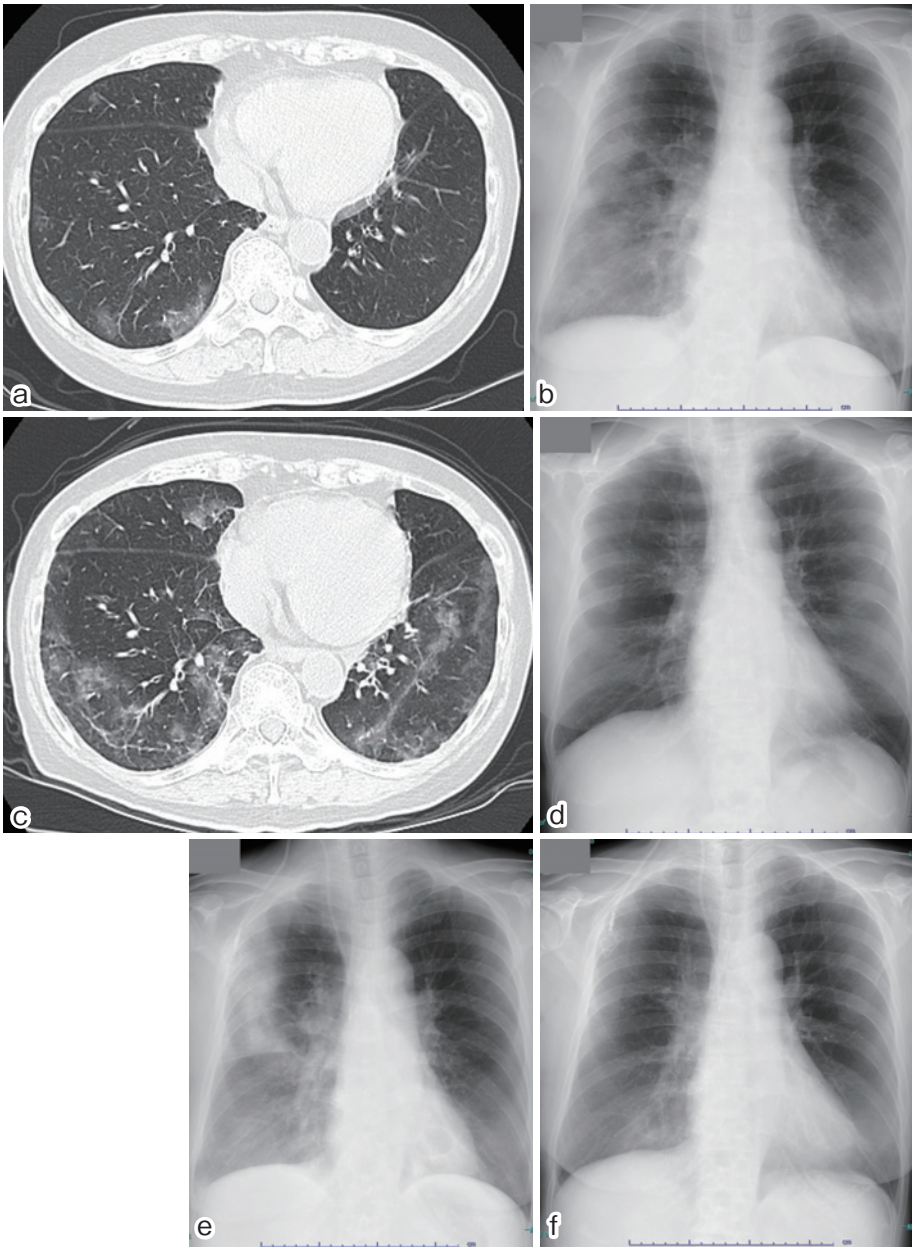
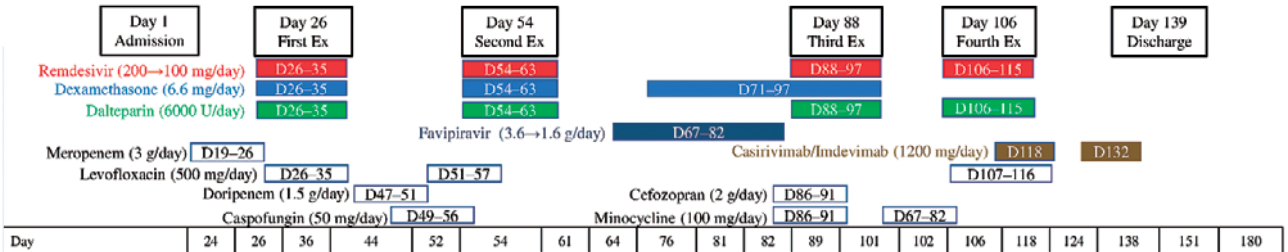


Figure 1 **(a)** Chest computed tomography scan sliced by lower lung field level on admission (Day 1 of illness). Diffuse ground glass opacities were detected in the right lower lobe. **(b)** Chest X-ray on Day 26 of illness. Ground glass opacities were detected in left and right lung fields. **(c)** Chest computed tomography scan sliced by lower lung field level on Day 26 of illness. Compared with chest computed tomography scan on admission, diffuse ground glass opacities in the left and right lung fields were more prominent. **(d)** Chest X-ray on Day 41 of illness. Compared with chest X-ray on Day 26 of illness, ground glass opacities in the left and right lung fields had lessened. **(e)** Chest X-ray on Day 54 of illness. Compared with chest X-ray on Day 41 of illness, ground glass opacities in the left and right lung fields had worsened. **(f)** Chest X-ray on Day 61 of illness. Compared with chest X-ray on Day 54 of illness, ground glass opacities in the left and right lung fields had lessened.

the test results changed due to changes of the SARS-CoV-2 PCR measuring instruments. The patient was confirmed to have the second E484K mutation on Day 54. Several tests for IgM and IgG antibodies against SARS-CoV-2 (detection sensitivity: 0.2 AU/ml and 0.35 AU/ml, respectively) were performed, but the levels were below the detection limit in all instances. The B-cell lymphoma had not worsened either, as evidenced by the absence of superficial lymph node swelling or sIL-2R elevation.



Day	24	26	36	44	52	54	61	64	76	81	82	89	101	102	106	118	124	138	151	180
Body temperature (℃)	36.7	37.1	36.5	37.0	36.5	38.2	36.5	36.1	36.9	38.5	36.9	37.5	37.4	36.8	37.6	36.5	36.4	36.3	-	-
SpO_2 (%) O2 nasal (L)	98	97	98	98	98	98	97	99	96	98	98	95 (1)	97 (2)	95 (1)	96 (1)	99	98	100	98	98
WBC (10^3/μL)	-	2.3	1.9	-	-	6.3	-	3.0	-	-	4.6	1.3	-	-	1.1	-	1.8	1.9	2.3	2.2
Lymphocytes (/μL)	-	368	368	-	-	491	-	210	-	-	69	66	-	-	121	-	984	792	1380	1320
CRP (mg/dL)	-	4.9	0.18	-	-	11.12	-	0.53	-	-	-	8.56	-	-	12.18	-	0.33	<0.06	0.08	<0.06
CD4 (cells/μL)	-	-	-	-	-	-	-	-	-	-	15	-	-	-	-	-	116	-	223	192
IgM (mg/dL)	-	4.9	-	-	-	-	-	-	-	-	-	-	-	-	2.5	-	4.7	-	3.8	4.2
IgG (mg/dL)	-	477	-	-	-	-	-	-	-	-	-	-	-	-	522	-	468	-	468	404
sIL-2R (U/mL)	-	-	-	-	851	-	-	-	-	-	-	-	-	-	-	-	653	-	432	403
SARS-CoV-2 PCR	P	-	-	P Ct value; n1 (26.78) n2 (29.66)	-	P (E484K) Ct value; n1 (30.92) n2 (33.47)	-	-	P Cycle; 36/45	-	-	-	P Cycle; 35/45	-	-	-	-	P Cycle; 34/45	P Cycle; 34/45	N
SARS-CoV-2 IgM (AU/mL)	-	-	-	-	-	-	N.D	-	-	-	N.D	-	-	N.D	-	N.D	-	N.D	-	N.D
SARS-CoV-2 IgG (AU/mL)	-	-	-	-	-	-	N.D	-	-	-	N.D	-	-	N.D	-	N.D	-	N.D	-	N.D

Figure 2 Clinical course from admission (Day 1) to Day 180, including discharge on Day 139.
Ex; exacerbation, D; day, N.D.; not detected, -; not applicable, P; positive, N; negative, n1; SARS-CoV-2 N1 gene, n2; SARS-CoV2 N2 gene, WBC; white blood cells, CRP; C-reactive protein, CD4; CD4 T cells, IgG; immunoglobulin G, sIL-2R; serum soluble interleukin 2 receptor

Discussion

We treated our patient with four cycles of two-drug therapy with bendamustine and rituximab for stage IV B-cell lymphoma (indolent lymphoma) over a four-week period. Rituximab is an anti-CD20 monoclonal antibody whose mechanism of action primarily involves acting on CD20 on the surface of activated B cells that have become cancerous to suppress proliferation of neoplastic cells and reduce IgG production[3]. Bendamustine is classified as an alkylating agent with a nitrogen mustard and purine analog-like benzimidazole[4]. Its mechanism of action involves cytotoxic effects mediated by multiple mechanisms, including DNA damage by alkylation, p53-dependent and -independent induction of apoptosis, and induction of mitotic catastrophe by inhibition of the M checkpoint of the cell cycle[4]. A recent case report described a patient who was immunocompromised due to bendamustine therapy and had prolonged SARS-CoV-2 infection despite ultimately achieving a negative PCR result[5]. In our patient, the clinical findings and course indicated no relapse of B-cell lymphoma. Consequently, considering that our patient had both a low $CD4^+$ T-cell count and low IgG, indicating suppression of both cell-mediated and humoral immunity, we believe that the effects of bendamustine therapy were the most reasonable explanation for why she remained immunocompromised for such a long period. Therefore, she was considered to have persistent SARS-CoV-2 infection and the SARS-CoV-2 PCR test did not become negative.

On the other hand, since the number of $CD4^+$ T-cell increased, it is possible that SARS-CoV-2 PCR became negative on Days 180 due to the improvement of cell-mediated immunity. Nevertheless, SARS-CoV-2 infection in immunocompromised patients is a medically crucial issue.

Another recent case report described a patient treated with rituximab and several immunosuppressants for severe antiphospholipid syndrome who developed persistent SARS-CoV-2 infection that led to severe pneumonia and death[6]. Cytokine storm is believed to be one cause of severe pneumonia and death in patients infected with SARS-CoV-2[7]. Our patient ex-

perienced repeated episodes of moderate pneumonia that never became severe but did become difficult to treat. The reason for this could be that she did not have a high-level cytokine storm because we were able to treat her for SARS-CoV-2 soon after onset, and also that she was severely immunocompromised due to receiving four cycles of two-drug therapy with bendamustine and rituximab. Furthermore, it is possible that the observed severity of the SARS-CoV-2 infection was the result of having the E484K mutation.

To treat COVID-19, it is currently considered important to treat both infected cells and extracellularly released viruses[1]. The COVID-19 treatments we used in our patient included steroids to reduce inflammation[8], remdesivir to inhibit RNA polymerase involved in viral replication[9], and the nucleoside analogue/RNA-dependent RNA polymerase inhibitor favipiravir[10]. Remdesivir and steroids did have some effect. However, they did not fully cure the pneumonia because it would relapse whenever the drugs were stopped. We also attempted favipiravir monotherapy, but it had no effect whatsoever on the pneumonia. We had hoped that the patient would begin producing neutralizing antibodies against the SARS-CoV-2 spike protein during treatment with these drugs, but we presume that this did not occur due to her immunocompromised state. One new treatment for SARS-CoV-2 is a two-drug antibody cocktail of casirivimab and imdevimab, which became covered by Japanese National Health Insurance on July 19, 2021 for the indication of mild to moderate I severity detected early and not requiring oxygen therapy[11]. Casirivimab and imdevimab inhibit viral replication by binding to the spike protein on the surface of SARS-CoV-2 and preventing the virus from invading cells[10]. It is hoped that using two different antibodies will make the treatment more effective against mutant viruses[11]. Our patient was unlikely to produce antibodies against SARS-CoV-2 and was experiencing repeated episodes of pneumonia due to long-term persistent infection. For this reason, and with consideration to the possibility that her B-cell lymphoma might worsen in the future, we decided to try antibody cocktail therapy to shorten her hospital stay. After antibody cocktail therapy, the patient exhibited no symptoms of COVID-19 and her laboratory and imaging results did not indicate worsening infection, but her PCR test result remained positive. This indicates that although antibody cocktail therapy can stop virus released from infected cells from infecting uninfected cells, treatment to control the virus in infected cells is still necessary. In a person with normal immune function, killer cells from the innate immune system and natural killer cells from the acquired immune system attack and destroy cells infected by viruses[1]. It is also believed that cells infected with SARS-CoV-2 self-destruct by apoptosis[12]. Therefore, normal immune function is necessary to attack and destroy infected cells. It may also be necessary to establish special therapies for treatment in immunocompromised patients.

The realistic treatment approach for immunocompromised patients going forward will be a passive approach of waiting for immune function to improve while continuing antibody cocktail therapy as appropriate and adding available antivirals as necessary. In addition, early treatment of SARS-CoV-2 infection might also be important, including our case. It will be necessary to quickly develop SARS-CoV-2 RNA synthesis inhibitors that act directly on the virus inside of cells, as well as clinical applications of stem cell transplantation[13] and chimeric antigen receptor (CAR)-NK therapy to leverage the abilities of the immune system in such patients[14].

In conclusion, our case illustrates that SARS-CoV-2 PCR positive can persist for a very long time in severely immunocompromised patients. These patients are at risk of new concomitant infections or worsening of existing infection, making it extremely important to establish new therapeutic agents for SARS-CoV-2.

Authors' contributions

H. Hirokawa and H. Hirata wrote the manuscript. Since R.K, M.I, T.I, T.H, J.S, and A.O are involved in the medical care of many COVID-19 patients, they have specialized knowledge, they provided advice in writing the dissertation. S.T is a professor of respiratory medicine with a core medical institution in the region, and he examines many COVID-19 patients and provided advice because he has a wealth of knowledge. M.A checked the proofreading and English translation of the dissertation. K.Haruki and Y.F were directly involved in the medical care of this patient. As a

specialist in blood diseases in the case, T.O, H.T, and K.Hashimoto were directly involved in medical treatment. All authors read and approved the final manuscript.

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