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## Note

## No SARS-CoV-2 RNA detected in the convalescent plasma of COVID-19 patients with different disease severity



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## ARTICLE INFO

## Article history:

Received 2 November 2020

Received in revised form

8 December 2020

Accepted 10 January 2021

Available online 15 January 2021

## Keywords:

Coronavirus 2019

Severe acute respiratory syndrome coronavirus 2

RNA

Convalescent plasma

Passive immunization

## ABSTRACT

**Introduction:** Convalescent plasma transfusion (CPT), a potential therapy for coronavirus disease 2019 (COVID-19), requires strict quality control of the donor blood. Whether to confirm the disappearance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA (RNAemia) in convalescent donor blood or not is unclear. Reports recommending the proof of viral disappearance from the blood are controversial. Foreseeing CPT in treating COVID-19 patients in Japan, we investigated RNAemia in 100 convalescent donors with mild, moderate, and severe COVID-19.

**Methods:** Between April 30 and July 30, 2020, we measured RNAemia in the plasma samples of donors with resolved COVID-19. Data on patients' demographics, comorbidities, pneumonia, treatment, and real-time polymerase chain reaction results for SARS-CoV-2 were collected. Date of onset of initial symptoms or date of positive testing (for asymptomatic patients) were self-reported by the patients. Disease severity was defined as: no, mild, moderate oxygen demand, or severe (requiring mechanical ventilation).

**Results:** Of 100 donors (58 males [58.0%]; median age, 47 [range 22–69] years) screened as of July 30, 2020, 77 (77.0%); 19 (19.0%); and 4 (4.0%) had mild, moderate, and severe disease, respectively. Median time between onset and testing was 68.5 (range, 21–167) days. SARS-CoV-2 RNA was not detected in any of the plasma samples.

**Conclusion:** RNAemia was not found in recovered COVID-19 patients at least 21, 27, and 57 days after the onset of mild, moderate, and severe symptoms. Our study may contribute to determining a suitable time for collecting convalescent plasma from COVID-19 patients and to future CPT use.

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**Abbreviations:** COVID-19, coronavirus disease 2019; CPT, convalescent plasma transfusion; FDA, the U.S. Food and Drug Administration; JRC, Japanese Red Cross Society; NCGM, National Center for Global Health and Medicine; NIID, National Institute of Infectious Disease; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SNS, social networking services.

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<https://doi.org/10.1016/j.jiac.2021.01.004>

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## 1. Text

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a global health concern. Convalescent plasma transfusion (CPT) is a passive immunization therapy that uses neutralizing antibodies collected from recovering patients. On August 23, 2020, the U.S. Food and Drug Administration (FDA) authorized the emergency use of investigational convalescent plasma for treating COVID-19 patients [1]. The efficacy of CPT in treating COVID-19 has

been reported in case series in China, Korea, and the U.S [2,3]. A randomized controlled trial conducted in China revealed that adding CPT to the standard of care reduced the time taken for clinical improvement (within 28 days); however, the study was underpowered [4]. Under the given circumstances, we designed the collection of convalescent plasma and determination of its anti-SARS-CoV-2 spike protein antibody titer including the neutralizing activity, foreseeing its use in treating COVID-19 in Japan. While strict quality control of donor blood is important in CPT, little is known about the persistence of SARS-CoV-2 RNA (RNAemia) in the convalescent plasma of donors. While some reports recommend proof of viral disappearance from the blood before CPT [5], the FDA does not recommend viral screening in asymptomatic donors after 14 days of symptom resolution [6], because there has been no report of blood transmission of SARS-CoV-2, and blood transfusion from donors in pre-symptomatic phase did not transmit the virus [7]. For the safe use of convalescent plasma, we aimed to investigate RNAemia in 100 blood donor samples.

Transfusion product procurement in Japan was launched by the National Center for Global Health and Medicine (NCGM) in Tokyo, in collaboration with the Japanese Red Cross Society (JRC) and the National Institute of Infectious Disease (NIID). NCGM and NIID have been pivotal organizations in Japan's response to COVID-19. The former is a tertiary care national hospital, and the latter is a government-managed national research institution. JRC, the blood banking organization in Japan, plays a key role in the blood donation scheme. In this study, the NCGM took responsibility for the patients' recruitment, collected data on the patients' demographics, and finally confirmed the eligibility of the patients for blood donors. The NIID conducted real-time polymerase chain reaction (RT-PCR) assay for SARS-CoV-2 detection in the plasma to rule out RNAemia in patients.

We initially recruited patients from our hospital and then expanded to external resources. Accessing only to patients who were admitted to NCGM did not suffice the number of donors we needed, and we approached patients from outside sources. On reaching out to external resources, social networking services such

as Facebook and Twitter were used. External cooperating medical institutions, where posters and leaflets were placed to recruit blood donors, were also approached. Our activities were covered by Japanese television stations, and thus audiences volunteered to participate in the study.

Between April 30 and July 30, 2020, we measured RNAemia in the plasma samples of donors with resolved COVID-19 (COVID-19 was previously confirmed in each patient by the presence of SARS-CoV-2 RNA in their respiratory specimens). Information on patient demographics, comorbidities, presence of pneumonia, treatment, and results of RT-PCR for SARS-CoV-2 in the plasma was collected. The date of onset was determined based on the patients' self-reported initial symptoms, or the date of positive testing if they were asymptomatic. Disease severity was defined as follows: a patient without oxygen demand, mild; a patient with oxygen demand but not a ventilator, moderate; and a patient requiring a ventilator to treat respiratory failure, severe. The study protocol was approved by the institutional review board (approval no.: NCGM-G-003536-01), and written informed consent was obtained from each patient.

Of 100 donors (males, 58 (58.0%); median age, 47 [range 22–69] years) screened as of July 30, 2020, 77 (77.0%); 19 (19.0%); and 4 (4.0%) had mild, moderate, and severe disease, respectively. Median time between onset and testing was 68.5 (range, 21–167) days (Table 1).

To increase the sensitivity of RT-PCR for detecting SARS-CoV-2 RNA, we used the maximum volume and concentration of RNA. RNA was extracted from 2-mL plasma using the QIA Symphony DSP virus/pathogen midi kit (Qiagen, GmbH), with a minimum elution volume of 60 µL; then, 25 µL of extracted RNA was added to the RT-PCR mixture with a total volume of 60 µL. Additionally, to increase the specificity of the RT-PCR, SARS-CoV-2 RNA was confirmed using two RT-PCR methods: 2019-nCoV\_N2, developed by the Centers for Disease Control and Prevention [8], and a probe generated in-house [9]. The method was constructed to detect SARS-CoV-2 RNA in plasma at the concentration of 100 copies/mL reproducibly. SARS-CoV-2 RNA was not detected in any of the plasma samples.

**Table 1**  
Patient demographics, comorbidities, presence of pneumonia, treatment, and results of SARS-CoV-2 RT-PCR in the convalescent plasma by disease severity (n = 100).

Variables	Disease severity		
	Mild (n = 77, 77.0%)	Moderate (n = 19, 19.0%)	Severe (n = 4, 4.0%)
<b>Demographics</b>			
Median age, years (range)	45 (21–167)	55 (31–67)	63.5 (52–69)
Males	40 (51.9%)	16 (84.2%)	2 (50.0%)
<b>Comorbidities</b>			
Hypertension	11 (14.3%)	9 (47.4%)	0 (0%)
Diabetes mellitus	5 (6.5%)	4 (2.1%)	1 (25.0%)
Dyslipidemia	11 (14.3%)	6 (3.2)	0 (0%)
Chronic obstructive pulmonary disease	1 (0.01%)	0 (0%)	0 (0%)
Cardiovascular disease	0 (0%)	0 (0%)	0 (0%)
Presence of pneumonia by imaging studies	0 (0%)	19 (100.0%)	4 (100.0%)
<b>Supportive therapy</b>			
Oxygen administration	0 (0%)	19 (100.0%)	4 (100.0%)
Mechanical ventilation	0 (0%)	0 (0%)	4 (100.0%)
ECMO	0 (0%)	0 (0%)	2 (50.0%)
<b>Medications</b>			
Steroid	0 (0%)	8 (42.1%)	2 (50.0%)
Lopinavir/ritonavir	0 (0%)	2 (10.5%)	2 (50.0%)
Favipiravir	0 (0%)	3 (15.8%)	1 (25.0%)
Hydroxychloroquine	0 (0%)	5 (26.3%)	0 (0%)
Remdesivir	0 (0%)	4 (21.1%)	0 (0%)
Tocilizumab	0 (0%)	1 (10.5%)	0 (0%)
Days from disease onset to the test (range)	74 (21–167)	65 (27–102)	98.5 (57–162)
RT-PCR in the plasma	UND	UND	UND

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ECMO, extracorporeal membrane oxygenation; RT-PCR, real-time polymerase chain reaction; UND, undetected.

Prolonged viral persistence is a concern in donors recovering from COVID-19, with one report indicating the presence of SARS-CoV-2 RNA in a patient's plasma 40 days after recovery from COVID-19-like symptoms. However, the infectivity of the virus was unknown [10]. Another study reported SARS-CoV-2 RNA detection during routine screening of healthy donors during the COVID-19 outbreak in Wuhan, China [11]. Such reports raise concerns about the appropriate timing of the plasma collection from COVID-19 patients for performing CPT and the need to screen for RNAemia. Our study screened for the presence of SARS-CoV-2 RNA in plasma using a retrospective cohort stratified by mild to severe disease; no patient, regardless of disease severity, was found positive—at least 21 days after disease onset. This result provides insight into the safer timing of plasma collection from donors after recovery from COVID-19.

There are some limitations to this study. First, the time of screening for RNAemia differed among these patients with different disease severity because patients with mild symptoms were likely to be discharged earlier and, hence, were included earlier in the study than were those with severe symptoms. Therefore, we could not compare the RNAemia results between patients in the early stages of recovery and the critically ill patients. The time between recovery and plasma collection was not determined because it was difficult to ascertain the timing of patient recovery. Next, comorbidities such as active cancer or an immunocompromised status were not included in the data collected in this study; therefore, the duration of RNAemia in these patients could not be confirmed. Finally, we could not adequately evaluate the persistence of RNAemia in severe cases. According to a prior report assessing longitudinal trends in plasma samples of COVID-19 patients, a patient requiring critical care had RNAemia up to 20 days post symptom onset [12]. Since our severe cases were small in size (4 cases) and were measured more than 50 days after the onset of the disease, more careful assessment about the persistence of RNAemia could be necessary in severe cases. Therefore, another study on the frequency of RNAemia in recovering patients in severe cases is desired.

In conclusion, we did not observe RNAemia in recovered COVID-19 patients, at least 21 days, 27 days, and 57 days after the onset of mild, moderate, and severe symptoms. Our study may contribute to determining a suitable time for collecting convalescent plasma from COVID-19 patients and to future CPT use.

#### Authors' contribution

All authors meet the ICMJE authorship criteria. HN organized the study and wrote the original draft. SK and SS conceived and designed the analysis. KO, MK, KT, EI, and IH were responsible for laboratory analysis. MT collected the data and edited the manuscript. NK, ME, TS, YM, TN, and MI supervised the manuscript. NO was responsible for the study. All authors read and approved the final manuscript.

#### Funding

This work was supported by the Health, Labor, and Welfare Policy Research Grants, Research on Emerging and Reemerging Infectious Diseases and Immunization (grant number 20HA1006).

#### Declaration of competing interest

None.

#### Acknowledgements

We thank all the clinical staff at the NCGM for their dedicated clinical practice and patient care.

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