

BRIEF REPORT

Persistent viral RNA shedding after COVID-19 symptom resolution in older convalescent plasma donors

William R. Hartman¹  | Aaron S. Hess^{1,2} | Joseph P. Connor² 

¹Department of Anesthesiology, University of Wisconsin-Madison School of Medicine and Public Health, Madison, Wisconsin

²Department Pathology and Laboratory Medicine, University of Wisconsin-Madison School of Medicine and Public Health, Madison, Wisconsin

Correspondence

William R. Hartman, Department of Anesthesiology, University of Wisconsin-Madison, School of Medicine and Public Health, 600 Highland Ave., B6/319, Madison, WI 53792, USA.
Email: wrhartman@wisc.edu

Abstract

Introduction: The novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is responsible for a worldwide pandemic. While the medical community understands the mode of viral transmission, less is known about how long viral shedding occurs once viral symptoms have resolved. Our objective was to determine how long the SARS-CoV-2 remains detectable following self-reporting of viral symptom resolution.

Methods: This study was approved by the University of Wisconsin Institutional Review Board. A cohort of patients who were previously SARS-CoV-2 positive less than 28 days after self-reported symptom resolution were retested for proof of viral recovery by nasal swab reverse transcriptase polymerase chain reaction for SARS-CoV-2 RNA.

Results: A total of 152 potential participants were screened, of which 5 declined, 54 were ineligible, and 93 were recruited; 86 of 93 completed testing. Eleven of 86 (13%) were still positive at a median of 19 days (range, 12-24 days) after symptom resolution. Positive participants were significantly older than negative participants (mean, 54 years; 95% confidence interval [CI], 44-63 vs 42 years; 95% CI, 38-46; $P = .024$). C_T values were significantly, inversely associated with age ($\beta = -.04$; $r^2 = 0.389$; $P = .04$). The number of days since symptom recovery was not apparently different between positive and negative participants.

Conclusion: We found evidence of persistent viral shedding in nasopharyngeal secretions more than 2 weeks after resolution of symptoms from confirmed COVID-19 infection. Persistent shedding was more common in older participants, and viral load was higher among older positive participants. These results underscore the necessity of testing COVID-19 convalescent plasma donors less than 28 days after symptom resolution.

Abbreviations: CI, confidence interval; C_T , threshold cycle; COVID-19, coronavirus disease 2019; IQR, interquartile range; NAT, nucleic acid testing; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; UW, University of Wisconsin.

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over 4.8 million people worldwide and caused over 310 000 deaths, with over 1.5 million infections and 90 000 deaths in the United States.^{1,2} Proving resolution of disease is critical for population health,

but this is limited by availability and uncertain interpretation of SARS-CoV-2 serology and the uncertain natural history of viral shedding detected by nucleic acid testing (NAT). A study in China found viral RNA in the stool of 17% of symptom-free patients 9 to 16 days after onset of disease.³ Systematic retesting has been limited in the United States.

2 | METHODS

This study was approved by the University of Wisconsin (UW) Institutional Review Board. Patients with previously laboratory-confirmed coronavirus disease 2019 (COVID-19) were screened as possible convalescent plasma donors. Information on the donor program was made available to all UW health providers via email and a hospital COVID-19 response dashboard and to the general public via the UW Health Web site and through community appeals in the local media. Potential donors were then able to self-identify via a local and toll-free phone hotline or email address that was managed by the U W's Office of Clinical Trials research coordinator staff. Initial screening was done using a scripted phone interview conducted by Office of Clinical Trials staff to verify primary infection and to determine symptom-free interval. Primary infection testing was performed at multiple sites with use of a variety of polymerase chain reaction (PCR)-based tests. Positive results were verified directly in the electronic medical record or via subject-provided hard copy of test results. Eligible donors were required to be symptom free by self-report a minimum of 14 days before donation. Participants reporting greater than 28 symptom-free days were eligible to donate without further testing. Those potential donors who reported being symptom free at least 14 days but less than 28 days were required to undergo repeat testing. For these cases, nasopharyngeal swabs were collected in viral media by trained nurses at an established UW Health drive-through testing facility. RNA was extracted with a kit (Maxwell RSC kit, Promega). SARS-CoV-2 N and RdRP sequences were amplified using an assay kit (Allplex 2019-nCoV Assay Kit, Seegene) on a reverse transcriptase PCR system (QuantStudio 5, Applied Biosystems). C_T values <40 for N- and/or RdRP were considered positive. Median C_T for all positive sequences was reported. Participants who underwent repeat testing were divided into two groups for analysis based on a positive or negative test result. Comparisons between groups were made with parametric and nonparametric statistics, as appropriate, and linear regression was used to compare continuous variables. Analyses were performed with commercial statistical software (Enterprise Guide 7.1, SAS Institute).

TABLE 1 Screening results for previously COVID-19-positive patients recruited to a convalescent plasma program (n = 86)

	SARS-CoV-2 viral RNA PCR result		P
	Positive (N = 11)	Negative (N = 75)	
Days since last symptoms, median (interquartile range)	19 (14-22)	18 (15-21)	.771 ^a
Age, mean ± SD	53 ± 15	43 ± 16	.024 ^b

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aWilcoxon-Mann-Whitney test.

^bt test.

3 | RESULTS

A total of 152 potential participants were screened, of which 5 declined, 54 were ineligible, and 93 were recruited. The most common reason for ineligibility was lack of laboratory-confirmed prior infection. Of participants recruited, 86 of 93 completed testing. Eleven of 86 (13%; 95% confidence interval [CI], 7%-22%) were positive, and 75 of 86 (87%; 95% CI, 80%-94%) were negative. Median positive C_T was 37.3 (interquartile range [IQR], 35.3-38.6). The range of days since symptom resolution was 12 to 24 among participants who tested positive and 11 to 30 among participants who tested negative. Positive and negative participants were not significantly different with respect to days since resolution of COVID-19 symptoms (median, 19 days; IQR, 14-22 vs 18 days; IQR, 15-21; $P = .771$) or female sex (63% vs 90%; $P = .090$). Positive participants were significantly older than negative participants (mean, 54 years; 95% CI, 44-63 vs 42 years; 95% CI, 38-46; $P = .024$). C_T values were significantly, inversely associated with age ($\beta = -.04$; $r^2 = 0.389$; $P = .04$). Eight of 11 positive participants were retested 6 or 7 days later, at a median of 22 days (IQR, 19-28) after resolution of symptoms. The remaining three positive participants passed the 28-day threshold before retesting. No negative participants were retested.

4 | DISCUSSION

We do not know if or for how long a patient remains contagious after COVID-19 symptoms resolve. Presymptomatic patients shed infectious virions.⁴ Previous studies have detected SARS-CoV-2 RNA in respiratory secretions 13 to 29 days after symptom onset.⁵ Patient isolation measures are dependent on assumptions about duration of viral shedding. We found that among

86 persons with prior laboratory-confirmed COVID-19 screened for a convalescent plasma donation, 13% still had detectable viral RNA in their nasopharyngeal secretions at a median of 19 days after symptom resolution. Median C_T values indicate that the viral RNA levels are near many commercial assays' limit of detection.⁶ Positive participants were significantly older than negative participants, and older participants had significantly higher viral RNA loads at time of testing. These data have limitations. Detectable RNA in secretions is not the same as shedding infectious virions, although NAT is the only rapid and widely available method for detecting SARS-CoV-2. There are likely biases and confounding, although the nearly identical time-to-test between groups suggests that positive results are not biased by earlier testing. These data demonstrate that patients with COVID-19 continue to shed detectable viral RNA for 2 to 4 weeks after resolution of symptoms. Efficient testing to confirm resolution of SARS-CoV-2 for surveillance and convalescent plasma programs should likely focus on patients beyond the 28-day window as the population of survivors in this time frame increases over time (Table 1).

ORCID

William R. Hartman  <https://orcid.org/0000-0003-2118-7591>

Joseph P. Connor  <https://orcid.org/0000-0002-1799-8335>

REFERENCES

1. Holshue ML, DeBolt C, Lindquist S, et al. First case of 2019 novel coronavirus in the United States. *N Engl J Med*. 2020;382(10):929–936.
2. Johns Hopkins University. COVID-19 Dashboard. Coronavirus Resource Center. Center for Systems Science and Engineering at Johns Hopkins University; 2020. <https://coronavirus.jhu.edu/map.html>. Accessed April 23, 2020.
3. Ling Y, Xu SB, Lin YX, et al. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. *Chin Med J (Engl)*. 2020;133:1039–1043.
4. Rothe C, Schunk M, Sothmann P, et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *N Engl J Med*. 2020;382:970–971.
5. Zheng S, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: retrospective cohort study. *BMJ*. 2020;369:m1443.
6. Lieberman J, Pepper G, Naccache SN, Huang M, Jerome KR, Greninger AL. Comparison of commercially available and laboratory developed assays for *in vitro* detection of SARS-CoV-2 in clinical laboratories. *J Clin Microbiol*. 2020. <https://doi.org/10.1128/JCM.00821-20>

How to cite this article: Hartman WR, Hess AS, Connor JP. Persistent viral RNA shedding after COVID-19 symptom resolution in older convalescent plasma donors. *Transfusion*. 2020;60:2189–2191. <https://doi.org/10.1111/trf.15927>