

Recurrence of Positive SARS-CoV-2 Viral RNA in Recovered COVID-19 Patients During Medical Isolation Observation

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Research Article

Keywords: SARS-CoV-2, recurrence, recovered patients, antibody detection

Posted Date: April 13th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-22829/v1>

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Version of Record: A version of this preprint was published on July 17th, 2020. See the published version at <https://doi.org/10.1038/s41598-020-68782-w>.

Abstract

Recently, the recurrence of positive SARS-CoV-2 viral RNA in recovered COVID-19 patients get more attention. Here we report a cohort study on the follow up of 182 recovered patients under medical isolation observation. There are 20 (10.99 %) patients out of the 182 were detected SARS-CoV-2 RNA turned positive, but none of them shows any clinical symptomatic recurrence indicating that COVID-19 has a good prognosis. Females and young patients aged under 15 have higher re-positive rate than the average, and none of the severe patients turned re-positive. Notably, most of the re-positive cases turn negative in the followed tests, suggesting that the importance of dynamic surveillance of SARS-CoV-2 RNA for infectivity assessment.

Introduction

At present, the pneumonia epidemic caused by SARS-Cov-2 has been spreading to other 159 countries with more than 850 thousand confirmed cases and up to 43, 000 deaths (World Health Organization, 2020), drawing a high-level concern over the world. Previous studies mainly focus on the clinical and Epidemiological characteristics of patients infected with SARS-CoV-2¹⁻³. With the increase of discharged patients, the follow-up and detection are particularly important. Previous studies found that, there are still positive RT-PCR test results in patients recovered from COVID-19⁴⁻⁶, and this finding has challenged the current hospital discharge criteria and containing of the pandemic. But rare cohort study about SARS-CoV-2 viral RNA re-positive in recovered COVID-19 patients was published. This report has analyzed 182 recovered COVID-19 patients of their SARS-CoV-2 viral RNA testing results during the 14 day's medical isolation observation period.

Methods

Since February 21st, COVID-19 patients of Shenzhen city who met all of the hospital discharge criteria were requested to stay in medical isolation observation for another 14 days, and the discharge criteria include:

1. Temperature below 37 degrees lasting at least 3 consecutive days;
2. Resolved respiratory symptoms;
3. Substantially improved in chest lesions computed tomography (CT) images, and
4. 2 consecutively negative RT-PCR test results with at least 1 day interval⁷.

RT-PCR Analysis

We have taken their nasopharyngeal swabs and anal swabs on the 7th and 14th day of the observation, to do the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) tests in Shenzhen Center for Disease Control and Prevention (CDC), and decide if they are allowed to go home. The RT-PCR tests were

performed on nasopharyngeal swab and anal swab by CDC followed with The RNA isolation kit (Roche) and RT-PCR test kits (Bio-Germ), which is described previously⁸. In briefly, we put nasopharyngeal swabs/anal swabs into a collection tube with 150 µL of virus preservation solution. Then 40 µL of cell lysate was taken to do 10s vortex followed by a 10 mins standing at room temperature. Then we collected the suspension after a 10 mins centrifugation with 1,000 rpm/min. Two target genes of SARS-CoV-2, including open reading frame 1ab (ORF1ab) and nucleocapsid protein (N), were simultaneously amplified and tested during RT-PCR assay. Target 1 (ORF1ab): forward primer CCCTGTGGGTTTTACTTAA; reverse primer ACGATTGTGCATCAGCTGA; and the probe 5'-VIC-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3'. Target 2 (N): forward primer GGGGAACCTTCTCCTGCTAGAAT; reverse primer CAGACATTTTGTCTCAAGCTG; and the probe 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'. A cycle threshold value (Ct-value) less than 37 was defined as positive, and Ct-value no less than 40 was defined as negative. A medium load, more than 37 and less than 40, will be defined as weak positive, which requires further confirmation by retesting.

We collected all of the recovered and isolated for 7+ days COVID-19 patients of their RT-PCR testing information, and analyzed the re-positive tests results.

Antibody Detection and Laboratory Testing

The main results and indicators of epidemiology, demography, clinical manifestation, laboratory examination of 182 recovered patients with COVID-19 were collected and analyzed. The total immunoglobulin, IgA, IgG and IgM of 14 re-positive patients were tested on the 7th day by a SARS-CoV-2 testing kit (WANTAI BioPharm) based on Chemiluminescence method. The S/CO<1 indicated antibody negative result, and S/CO>1 indicated antibody positive result.

This study was approved by the Shenzhen Samii Medical Center institutional review board and written informed consent was waived in light of the urgent need to collect clinical data. We declare that these data do not involve any private information of patients. All methods were performed in accordance with the relevant guidelines and regulations.

Results

Among all the recovered and isolated, there are 182 of them has been re-tested for at least one time, 84 (46.2%) of the 182 were males and 98 (53.8%) were females, the average age was 46.4±17.1 (median 49, ranges 1-81); 39 (21.4%) had severe symptoms, 143 (78.6%) mild and moderate (Table 1). Few of them had shown different symptom during the medical isolation, and was eliminated from COVID-19 recur.

There are 20 (10.99 %) patients out of the 182 re-tested has found positive results, this rate is slightly lower than 14%, which is officially announced by Guangdong province⁹. Thirteen of the re-positive cases are females, much more than males (7 cases). Thirteen of them tested to be re-positive on the 7th day,

and another 7 on the 14th day; 14 were tested as nasopharyngeal swabs positive, and 6 were anal swabs positive, none has found both swabs positive (Table2).

All the re-positives were suggested an antibody detection and laboratory testing by taking their blood, 14 out of the 20 re-positives were collected. Total immunoglobulin, IgA, IgG shown a positive result for all 14 re-positives, 4 of them shown IgM negative meanwhile all 14 re-positives were SARS-CoV-2 antibody carrier. And there was no clear abnormality was found in blood routine laboratory test (Table 3).

The re-positives are transferred to designated infectious hospital for quarantine treatments, and again their RT-PCR testing results of blood, nasopharyngeal swabs and anal swabs were collected on the 1st, 4th and 7th day (some were taken on 2nd and 6th), we got results of the 14 cases, 5 of them was positive, and 1 of the 5 (case 8) were found positive in all three testing days. And also, there are 3 (case 2, case 4 and case 15) of the 14 were negative in all three testing days, none have found positive in blood tests (Figure 1A).

Meanwhile, we have noticed a particular case of an 8-year-old boy who has Hubei exposure history during 10-26 Jan and was tested to be re-positive for repeated times (Case 19). He returned from Hubei Journey to Shenzhen on January 26th, the grandpa of him confirmed with COVID-19 on 31st. As a close contact, a throat swab was performed to the whole families and the boy was confirmed to get COVID-19 on February 1st, then hospitalized. During his hospitalization, no fever or other symptoms was found. He reached the hospital discharge criteria (according to the 4th Trail edition) on February 15th, and was transferred to isolation hotel for further 14 days (15th-28th Feb), due to the positive result of anal swab test on February 14th. During hotel isolation, results on two test days (21st and 28th Feb) are negative, and thus he was allowed to go home. And on his return visit to hospital on March 2nd, anal swab test showed positive result again, and he was requested hospitalization for a second time, during the second hospitalization, test results on the two test days (5th and 7th Mar) were negative, then he was transferred to isolation observation for the second 14 days from March 8th, and in the routine tests on March 15th (Day 7 of second isolation observation), the anal swab test result showed positive again (Figure 1B). two followed tests had been collected, there was a positive result again (anal swab) on 4th day after transferred to designated infectious hospital (Figure 1A, case 19). It has been 35 days since his first recovery and discharge from hospital, we can still observe viral RNA positive in him although there's no clinical symptom.

Discussion

None of 182 discharged patients (under "Management of Diagnosis and Treatment of Novel Coronavirus Pneumonia Scheme Trial Version 7) showed any clinical symptomatic recurrence (fever, cough, respiratory tract disease, etc.) during the 14 days of medical isolation, it indicated that COVID-19 has a good prognosis.

On the 7th day and 14th day of discharge from hospital and transferred to isolation, we still found positive results from nasopharyngeal swabs (7.69%) and anal swabs (3.3%) (n=182), it suggested that the recovered ones might still be carrying virus. There's no report about re-positive cases' infectiousness, but it still calls for alert, we may need to reevaluate hospital discharge criteria, and the current patients' management system. Similarly with the previous studies^{4,5}, most of the re-positive cases turn negative in the followed tests, especially case 19, as an asymptomatic carrier, who got re-positive twice, and this suggested that we need to furtherly optimize the diagnosis and treatment scheme.

Furthermore, young patients aged under 15 have much higher re-positive rate than the average rate (4/20), and most of the positive results (3/4) were found in anal swabs, and this indicated that juveniles have longer detoxification period and higher risk of fecal-oral transmission^{10,11}. In this study, 21.4% of the 182 recovered patients in isolation were severe patients, but none of the severe patients turned re-positive. It assumed that stronger immune response has been triggered in severe patients to restrain the virus more effectively. Just as the same differences between genders in morbidity and severe rate¹⁻³, females have higher re-positive rate and the contrast was much remarkable (13 females vs 7 males), it might concern gonadal hormone or physiological cycle, we cannot conclude it currently, and this could be a further research orientation.

The total immunoglobulin, IgA and IgG were all positive in all 14 re-positive patients, IgM was the emergency antibody, and it was also normal in the recovery period. The inflammation related tests of IL-6, CRP, SAA, PCT and thyroid functions were not abnormal, suggesting that re-positive patients had no obvious disease progress and infectivity. At present, it is believed that RNA negative conversion generally takes 2-3 weeks, a recent study indicated that SARS-CoV-2 nucleic acid can persist in feces for nearly 50 days¹². While the time from admission to discharge is shorter in patients with viral RNA re-positive (Table 1), suggesting that the virus may not be completely eliminated due to its lighter symptoms and faster reaching the discharge standard.

Taken together, younger, female, non-severe patients have higher risk on recurrence of positive SARS-CoV-2 viral RNA *via* a RT-PCR test, but all age and gender groups were at risk of re-positive, no severe patients was detected as re-positive results in our report, but the results was not enough to prove that severe patients has no risk to re-positive, all the discharged patients should take medical observation and quarantine for at least 14 days.

Declarations

Acknowledgements

This study was financially supported by grants JCYJ20180302153611416 from the Science and Technology Planning Project of Shenzhen Municipality (CN). We thank Jia Zhao, Xiao-Peng Hu and Zhi-Wen Li for help in data collection, Yi-Fan Zhang and Xiao-Hui Wang (Shenzhen CDC) for helping with confirmation of the diagnosis of the viral infection.

Author Contributions

B.Y. and W. X. L. was responsible for data analysis and chart making and manuscript writing. Y.W.A., and C.W. participated in data collection and contributed to the production of Figure. Z. R. Y., K. Z. and Z. Y. L. performed RT-PCR test and data collection. Y.X.C. and J.C.W. followed up the patients and collected informed consent. S.S., J. C. W. and H.Q.L. drafted and edited the manuscript. All authors reviewed the manuscript and approved the submission.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interest.

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Tables

Table 1 Basic information of recovered COVID-19 Patients

	Re-positive(n=20)	negative(n=162)	P-Value
Total (n=182)	20	162	/
Severe cases (n=39)	0***	39	≤0.001
Wuhan exposure (n=75)	5	70	0.091
Time from onset to admission	5.1±4.8	4.5±4.0	0.577
Time from admission to discharge	20.8±7.1*	25.6±7.6	0.012
Basic disease combined	6	57	0.425
Hypertension	3	26	/
Diabetes	0	12	/
Hyperlipemia	0	2	/
Cardiovascular disease	2	10	/
Malignant tumor	0	5	/
hepatopathy	1	7	/
Lung disease	0	3	/
Male	7	77	0.206
Female	13	85	
Median of Age	41.5 (Range 1-72)	49 (Range 1-81)	/
Average of Age	39.9±20.1	47.2±16.6	0.073

Continuous variables were analyzed using one-way ANOVA test and the categorical variables were compared using Chi-square test. *P<0.05, ***P<0.001 vs negative group.

Table 2 Recurrence of positive SARS-CoV-2 viral RNA in recovered COVID-19 Patients

Case Number	Sex	Age	Day 7 check		Day 14 check	
			nasopharyngeal swab	Anal swab	nasopharyngeal swab	Anal swab
Case 1	Male	38	negative	negative	negative	positive
Case 2	Male	53	negative	negative	positive	negative
Case 3	Female	40	positive	negative	/	/
Case 4	Female	61	negative	negative	positive	negative
Case 5	Female	64	negative	negative	positive	negative
Case 6	Female	53	negative	negative	positive	negative
Case 7	Female	33	positive	negative	/	/
Case 8	Female	1	negative	positive	/	/
Case 9	Female	34	negative	positive	/	/
Case 10	Male	43	positive	negative	/	/
Case 11	Female	34	negative	positive	/	/
Case 12	Male	38	negative	positive	/	/
Case 13	Female	50	positive	negative	/	/
Case 14	Female	50	positive	negative	/	/
Case 15	Female	5	negative	positive	/	/
Case 16	Female	55	positive	negative	/	/
Case 17	Female	72	negative	negative	positive	negative
Case 18	Male	54	negative	negative	positive	negative
Case 19	Male	8	negative	positive	/	/
Case 20	Male	12	positive	negative	/	/

Due to technical limitations, table 3 is only available as a download in the supplemental files section.

Figures

A

Samples	Day1			Day4			Day7		
	N	A	B	N	A	B	N	A	B
Case 1#	-	-		-	-				
Case 2	-	-		-	-	-	-	-	
Case 3	±	-		-	±		-	-	-
Case 4	-	-	-	-	-	-	-	-	-
Case 5	+	-	-	-	-	-	-	-	-
Case 6	-	-		-	-				
Case 7	-	-		-	-				
Case 8	-	+		-	±		±	±	
Case 9	-	+		-	-		-	-	
Case 13#	-	-	-	-	-	-			
Case 14#	-	-	-	-	-	-			
Case 15	-	-		-	-		-	-	
Case 16	-	-		-	-				
Case 19	-	-		-	+				

N, nasopharyngeal swab; A, anal swab; B, blood;
 +, positive; -, negative; ±, weak positive;
 #, those samples checked at Day 2 and Day 6.

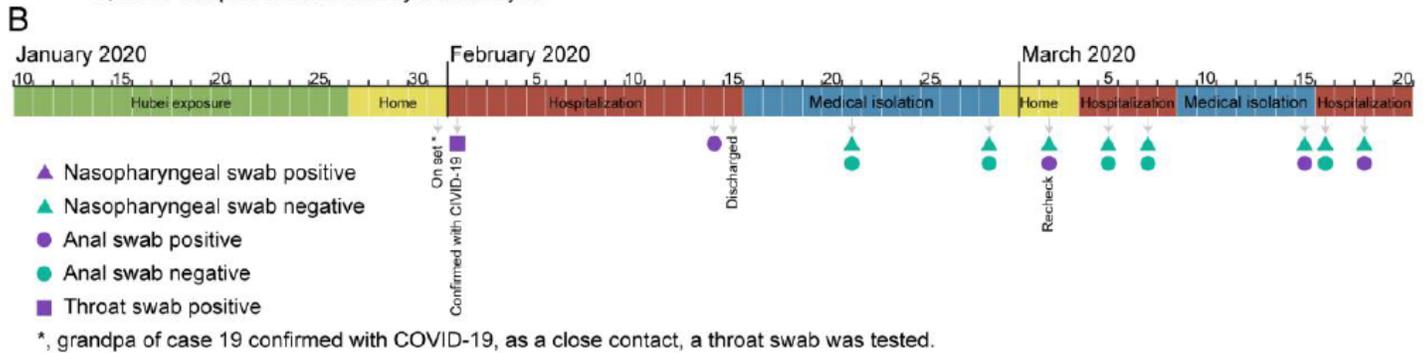


Figure 1

(A). RT-PCR testing of collected 15 re-positive cases out of 20. Data shown RT-PCR results of blood, nasopharyngeal swabs and anal swabs testing at 1st, 4th and 7th day (2nd and 6th day for case 1, 13 and 14). (B). The timeline of case 19.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table3.pdf](#)