

Characteristics of Recurrent Positive RT-PCR Findings of Recovered Patients From COVID-19 in Wuhan, China: A Retrospective study

Xiaohua Chen (✉ hannahchen2003@163.com)

Beijing Tropical Medicine Research Institute, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China <https://orcid.org/0000-0002-5700-705X>

Tie-Jun Shui

Yunnan Center for Disease Control and Prevention, Yunnan, 650011, China

Chao Li

Huangpi District Center for Disease Control and Prevention, Wuhan, 432200, China

Bi-ke Zhang

Chinese Center for Disease Control and Prevention, Beijing, 102206, China

Research Article

Keywords: COVID-19, Recurrent, reverse transcriptase- polymerase chain reaction

Posted Date: May 11th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-26257/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

After two month of the outbreak of corona virus disease 2019 (COVID-19) in Wuhan China, tens of thousands hospitalized patients, being recovered, little was known about the follow-up of the recovered patients. 758 recovered patients, hospitalized in 17 hospitals previously and quarantined in 32 rehabilitation post stations in Wuhan, China, were retrospectively reviewed for clinical characters, reverse transcriptase- polymerase chain reaction (RT-PCR) from throat swab specimens and serological COVID-19 rapid diagnostic test (RDT) for SARS-Cov-2. Among them, 59 patients (7.78%) had recurrent positive findings for COVID-19 on RT-PCR from throat swabs. Among the 50 patients which was effective samples for antibody detection, 45/50 (90.00%) and 4/50 (8.00%) patients had positive of IgG or dual positive of IgG/IgM RDT for COVID-19, respectively. A certain number of quarantined patients with COVID-19 had recurrent RT-PCR results of SARS-Cov-2, the possibility of transmission in the patients needs further investigation.

Introduction

The Coronavirus Disease 2019 (COVID-19) is an emerging respiratory infectious disease caused by SARS-CoV-2, which first occurred in early December 2019 in Wuhan, China. Until March 15, Covid-19 has affected 81,062 individuals in China, and 67041 were cured by multiple measures and aggressive treatment.

Following the national recommendations for diagnosis and treatment of pneumonia caused by 2019-nCoV (the 5th edition) and current status of clinical practice in Hubei Province, real-time reverse transcriptase–polymerase chain Reaction (RT-PCR) analysis was used to detect the causative virus from respiratory secretions [1,2]. The suspected patients for COVID-19 were confirmed diagnosed by positive result of RT-PCR for SARS-Cov-2, and 2 consecutively negative RT-PCR were as the criteria for hospital discharge. After that, the recovered patients with COVID-19 were quarantined at the rehabilitation post stations, the RT-PCR were performed tests for COVID-19 nucleic acid to determine if they could return to work.

Few studies have described positive RT-PCR test results in the patients recovered from COVID-19 [3-5] and the clinical characteristics of the recurrent RT-PCR results in recovered patients still unclear. Here, we studied the characteristics of recurrent positive results of RT-PCR for SARS-CoV-2 from recovered COVID-19 patients in Huangpi, Wuhan.

Methods

Data sources

We conducted a retrospective study focusing on the consecutive patients, confirmed diagnosis with COVID-19, hospitalized, then quarantined at the rehabilitation post stations from February 25, 2020, to March 15, 2020 from Huangpi district, Wuhan, China. The diagnosis of patients was based on the 5th edition of the National Guideline on Prevention and Control of the Novel Coronavirus Pneumonia (PC-NCP) published by the National Health Commission of China on February 8, 2020. [1]. The patients with a laboratory confirmed infection and had 2 consecutively negative RT-PCR test results separated by at least 1 day before hospital discharge were enrolled in. 758 patients with confirmed diagnosed of COVID-19 were hospitalized in 17 hospitals, then quarantined at 32 rehabilitation post stations in Huangpi district, Wuhan, China. The patient basic information, clinical type, and results of RT-PCR from throat swabs for COVID-19 were noted and analyzed. This study was approved by the ethics

committee of Yunnan Center for Disease Control and Prevention, Yunnan, China, and all patients gave written informed consent.

RT-PCR Detection of SARS-Cov-2

The real-time reverse transcriptase polymerase chain-reaction (RT-PCR) test, using throat swab specimens, was performed at Wuhan Ping an Hao Medical Laboratory according to the protocol of DAAN Gene Co.,Ltd of SUN Yat-sen university. SARS-CoV-2 open reading frame 1ab (ORF1ab), nucleocapsid protein (NP) genes fragments were amplified and the Conditions for amplifications were 50°C for 10 min, 97°C for 1 min, followed by 40 cycles of 97°C for 5 s and 58°C for 30 s. When two targets (ORF1ab, NP) tested positive by specific real-time RT-PCR, the case would be considered to be laboratory-confirmed. A cycle threshold value (Ct-value) less than 37 was defined as a positive test, and a Ct-value of more than 40 was defined as a negative test. A medium load, defined as a Ct-value of 37 to 40, required confirmation by retesting.

Serological COVID-19 RDT test

Serum was separated by centrifugation at 2500 g for 5 min within 24h of collection.

SARS-CoV-2 IgG/IgM RDT kit were from Innovita Biological Technology Co., Ltd. (Beijing, China). Briefly, the assay is performed by adding 10 µl of serum/plasma or 20 µl of whole blood, and 2 drops (80 µl) of the assay buffer. Reacting bands were read after 15 min and its density was determined as negative, and positive. The final results were agreed upon by 3 investigators.

Statistical analysis

All statistical analyses were performed using SPSS version 16.0 (SPSS Inc). Means for continuous variables were compared using independent-group t test when the data were normally distributed; otherwise, the Mann-Whitney test was used. The binary logistic regression analysis were performed. Probability (p) values less than 0.05 were considered significant.

Results

Epidemiological characteristics

By 15 March 2020, clinical data were collected on 758 patients quarantined at rehabilitation post stations in Huangpi, Wuhan, Hubei province with laboratory confirmed COVID-19 infection. Twenty one (2.77%) of the patients were aged <10 years, 19 (2.51%) were aged 10-19 years, 76 (10.02%) were aged 20-29 years, 148 (19.53%) were aged 30-39 years, 153 (20.18%) were aged 40-49 years, 158 (20.84%) were aged 50-59 years, 120 (15.83%) were aged 60-69 years, 50 (6.60%) were aged 70-79 years, 13 (1.72%) were aged 80 years and older. The median age was 48 years (interquartile range 35-58 years), and the mean (SD) age was 46.61 (16.82) years (Table 1). The 396 patients (51.40%) were male. All of 758 patients were confirmed diagnosis cases, and had 2 consecutively negative RT-PCR test results separated by at least 1 day before hospital discharge.

Of them, 59 patients (59/758, 7.78%) were detected positive RT-PCR test results when quarantined at rehabilitation post stations. Of the 59 patients with recurrent RT-PCR results for COVID-19 after hospital discharge, One (1.70%) of the patients were aged <10 years, 1 (1.70%) were aged 10-19 years, 9 (15.25%) were aged 20-29 years, 10 (16.95%) were aged 30-39 years, 5 (8.47%) were aged 40-49 years, 13 (22.03%) were aged 50-59 years, 13 (22.03%) were

aged 60-69 years, 4 (15.83%) were aged 70-79 years, 3 (5.09%) were aged 80 years and older. The median age was 52 years (interquartile range 35-65 years), and the mean (SD) age was 49.61 (16.64) years (Table 1). The 29 patients (49.15%) were male.

Table1. Clinical characters of Patients recovered from Coronavirus Disease 2019(COVID-19).

Measure	(as of March 15, 2020)	RT-PCR Results						Difference*	(95% CI)	P value
		Total		Positive		Negative				
		(n)	(%)	(n)	(%)	(n)	(%)			
		758	100.00%	59	7.78%	699	92.22%			
Age [years]	Minimum to Maximum	1-92	/	8-87	/	1-92	/	-3.257 ± 2.279	-7.731 to 1.217	0.1534
	Median (interquartile)	48	(35-58)	52	(35-65)	47	(35-57)			
	Mean±SD (95% CI)	46.61±16.82	45.41-47.81	49.61±18.64	44.75-54.47	46.35±16.65	45.12-47.59			
	≥80 years	13	1.72%	3	5.09%	10	1.43%			
	70-79years	50	6.60%	4	6.78%	46	6.58%			
	60-69years	120	15.83%	13	22.03%	107	15.31%			
	50-59years	158	20.84%	13	22.03%	145	20.73%			
	40-49years	153	20.18%	5	8.47%	148	21.17%			
	30-39years	148	19.53%	10	16.95%	138	19.75%			
	20-29years	76	10.02%	9	15.25%	67	9.59%			
	10-19years	19	2.51%	1	1.70%	18	2.58%			
<10years	21	2.77%	1	1.70%	20	2.86%				
Gender	Male	396	51.40%	29	49.15%	367	52.50%	-0.03351 ± 0.06780	-0.1666 to 0.09958	0.62
	Female	362	48.60%	30	50.85%	332	47.50%			
Severity of disease	Mild	229	25.12%	16	27.12%	213	30.47%	0.008608 ± 0.08114	-0.1507 to 0.1679	0.92
	Moderate	465	67.63%	40	67.80%	425	60.80%			
	Severe	60	6.67%	3	5.08%	57	8.16%			
	Critical	4	0.58%	0	0.00%	4	0.57%			
Time from disease onset to diagnosis (days)	Minimum to Maximum [days]	1-61	/	1-33	/	1-61	/	1.940 ± 1.054	-0.1289 to 4.008	0.07
	Median (interquartile) [days]	8	(3-13)	6	(1-11)	8	(3-14)			
	Mean±SD (95% CI) [days]	9.31±7.78	8.76-9.87	7.53±7.15	5.66-9.39	9.47±7.82	8.88-10.05			
	≤1 week	374	49.34%	34	57.63%	340	48.64%			
	≤2 weeks	218	28.76%	17	28.81%	201	28.76%			
	≤3 weeks	107	14.11%	5	8.47%	102	14.59%			
	≤4 weeks	39	5.15%	1	1.70%	38	5.44%			
	≤5 weeks	19	2.51%	2	3.39%	17	2.43%			
	≥8 weeks	1	0.13%	0	0.00%	1	0.14%			

Abbreviations:IQR,interquartile range;*Difference between (Negative - Positive) ± SEM;

Clinical features

The severity of disease was mild to critical for 758 patients, while mild to severe for 59 patients with recurrent positive result of RT-PCR for SARS-Cov-2. Among these 758 patients, there were 229 (25.12%) cases judged as moderate, 465 (67.63%) cases judged as moderate, 60 (6.67%) judged as severe, and 4 (0.58%) cases judged as critical, which was graded according to the guideline of PC-NCP [1] (Table 1). While of those, 16 of the 59 patients (27.12%) were judged as mild, 40 (67.80%) judged as moderate, 3 (5.08%) judged as severe, but 0 (0.00%) cases were judged as critical (Table 1).

For all 758 patients, The time range from onset of symptoms to first hospitalized ranged were 1-61 days. The median time from onset of symptoms to first hospital admission was 8 (3-13) days. And the mean (SD) was from onset of symptoms to first hospital admission was 9.31 (7.78) days. For 59 patients with recurrent positive result of RT-PCR for SARS-Cov-2, the time range from onset of symptoms to first hospitalized ranged were 1-33 days. The median time from onset of symptoms to first hospital admission was 6 (1-11) days. And the mean (SD) was from onset of symptoms to first hospital admission was 7.53 (7.15) days. To March 15, 2020, the patients remain asymptomatic and no more infections were detected (Table 1).

Recurrent positive results of RT-PCR for SARS-Cov-2

Of the 59 patients with recurrent positive results of RT-PCR for SARS-Cov-2, the time from onset of symptoms to last time of positive results of RT-PCR for SARS-Cov-2 was ranged from 14 to 61 days, The median time from onset of symptoms to first hospital admission were 30 (23-39) days, and the mean (SD) of those were 31.78 (12.17) days, respectively. In addition, the time from diagnosis to last time of positive results of RT-PCR for SARS-Cov-2 was ranged from 13 to 48 days, The median time and the mean (SD) from onset of symptoms to first hospital admission were 24 (20-29) days, and 25.53 (7.857) days, respectively. Last, the time from quarantined to last time of positive results of RT-PCR was ranged from 1 to 19 days, The median time and the mean (SD) from onset of symptoms to first hospital admission were 8 (4-11) days, and 7.746 (4.13) days, respectively (Table 2).

Table2.Characteristics of 59 recovered Patients From COVID-19 With positive results of RT-PCR.

	Positive RT-PCR Results			
	From disease onset		From Diagnosis	From hospital discharge
	First time	Last time	Last time	Last time
Median(IQR)	1 (-2-7)	30 (23-39)	24 (20-29)	8 (4-11)
Minimum to Maximum	-4-31	14-61	13-48	1-19
Mean±SD	4.153±7.701	31.78±12.17	25.53±7.857	7.746±4.13
95% CI of mean	2.146-6.159	28.61-34.95	23.48-27.54	6.67-8.822
Last (weeks)≥1 week (n)	/	/	/	23
≥2 weeks (n)	/	/	1	33
≥3 weeks (n)	/	11	15	3
≥4 weeks (n)	/	15	25	/
≥5 weeks (n)	/	9	12	/
≥6 weeks (n)	/	15	5	/
≥7 weeks (n)	/	3	3	/
≥8 weeks (n)	/	2	/	/
≥9 weeks (n)	/	4	/	/

Abbreviations:IQR,interquartile range;

Serological RDT test for the patients with recurrent positive results of RT-PCR for SARS-Cov-2

The IgG and IgM antibody for SARS-CoV-2 were tested in 50 of 59 COVID-19 patients with recurrent positive results of RT-PCR for SARS-Cov-2. 45 of 50 (90.00%) patients had positive results of IgG antibody for SARS-CoV-2, while 4 of 50 patients (8.00%) had positive results of dual IgM/IgG antibody for SARS-CoV-2.

No risk factor in the patients with recurrent RT-PCR results for SARS-CoV-2 infection were found

There were no significant difference between age, gender, severity, time between disease onset to diagnosis. Binary logistic regression analysis showed that not age, gender, severity of disease, nor time last from onset to hospitalized was the risk factor for recurrent RT-PCR in quarantined patients with COVID-19. Based on the current data, no risk factor in the patients with recurrent RT-PCR results for SARS-CoV-2 infection were found.

Discussion

Few previous investigations have evaluated follow-up RT-PCR results for SARS-Cov-2 in patients infected with COVID-19 [3,4,5]. A few reports have presumed asymptomatic Carrier for transmission of COVID-19[6]. Our investigation suggests that among cured patients with COVID-19, 7.78% (59/758) patients have recurrent positive results of RT-PCR for SARS-Cov-2, with most among patients with positive findings on IgG or IgG/IgM of SARS-Cov-2 RDT. These results suggest that recurrent positive results of RT-PCR for SARS-Cov-2 commonly appear in patients with COVID-19.

Our results show a low prevalence (7.78%; 59/758) of SARS-CoV-2 nucleotides recurrent positive results in throat swabs specimens of patients with COVID-19, whom were quarantined at the rehabilitation post stations, ranged from 1-19 days after quarantined. The results was consistent with previous study on positive RT-PCR test results in patients recovered from COVID-19, four patients with COVID-19 who met criteria for hospital discharge or discontinuation of quarantine in China (absence of clinical symptoms and radiological abnormalities and 2 negative RT-PCR test results) had positive RT-PCR test results 5 to 13 days later [3]. Other Two studies also reported that PCR Assays Turned Positive with 25 of 172 (14.5%) and 15 of 70 (21.4%) discharged patients from shenzhen [4] and Wuhan [5]. These findings confirmed again that there was a certain proportion of recovered patients may still experience a prolonged nucleic acid conversion regardless of the relief in symptoms or radiography.

Firstly, RT-PCR has been widely deployed in diagnostic virology and has yielded few false-positive outcomes[7]. The false-negative results have been observed related to the quality of the kit, the collected sample, or performance of the test [6]. In this study, the specimens from patients from 17 hospitals then quarantined in 32 rehabilitation post stations, and RT-PCR were performed by the trained professional in the powerful standard laboratory. Less likely for technical reasons result in recurrent positive results of RT-PCR for SARS-Cov-2.

Secondly, Several serological immunoassays have been developed by IVD companies for the detection of SARS-CoV-2viral proteins and antibodies in the serum or plasma. IgM can be detected in the patient samples from 10 to 30 days after SARS-CoV-2 infection, while IgG can be detected from 20 days onwards [8]. The IgM response occurs earlier than that of IgG, but it then decreases and disappears [9]. in this study, predominant recurrent patients (45/50, 90.00%) showed positive result of IgG for SARS-Cov-2, which may implied the patients had a protective reaction.

Thirdly, the asymptomatic carrier was presumed transmission of COVID-19 infection [6],The possibility of transmission in the recovered patients from COVID-19 with recurrent positive results of RT-PCR for SARS-Cov-2 needs further investigation.

Limitations of this study

Our study has several limitations. There was a small sample size of patients with COVID-19. A larger cohort and follow-up in more details would help to understand the characters of recurrent positive results of RT-PCR of the disease. In addition, as only the several basic information involved age, gender, severity of disease, time from onset of illness to diagnosis were collected, no risk factor was found in this study. It might be that more clinical features related to covid-19 enrolled in, the risk factors will be identified.

Conclusions

In this study, a certain number of quarantined patients with COVID-19 had recurrent RT-PCR results of SARS-Cov-2. Although there was a low prevalence of recurrent positive results of RT-PCR for SARS-Cov-2 in recovered patients with COVID-19, and most of them had protective role, the possibility of transmission in the patients needs further investigation.

Declarations

Ms Tiejun Shui is a deputy director of the physician in the Yunnan Center for Disease Control and Prevention, Yunnan, China. She mainly engaged in the field epidemic work of infectious diseases.

Ms Chao Li is a physician in the Huangpi District Center for Disease Control and Prevention, Wuhan, China. She mainly engaged in data statistics and processing of disease.

Funding: The authors received no external funding for this research.

Acknowledgments

We thank Wensheng Lei from Huangpi District Center for Disease Control and Prevention for data collection from recovered COVID-19 patients, and Chao Ma from Chinese Center for Disease Control and Prevention for advice for the manuscript.

Author Contributions

Conceived and designed the experiments: Tiejun Shui, and Bi-ke Zhang. Data collection: Tiejun Shui, Chao Li. Analyzed the data and wrote the paper: Chen XH.

Compliance with Ethical Standards

This study was approved by the Medical Ethics Committee of Yunnan Center for Disease Control and Prevention, Yunnan, People's Republic of China.

Competing Financial Interests

The authors declare that they have no conflicts of interest.

Ethical Approval

All the procedures in the studies involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. National Health Commission of the People's Republic of China. The guideline on diagnosis and treatment of the novel coronavirus pneumonia (NCP):revisedversion of the 5th Accessed February8,2020. <http://www.nhc.gov.cn/xcs/zhengcwj/202002/d4b895337e19445f8d728fcf1e3e13a.shtml>
2. Liu R, Han H, Liu F, Lv Z, Wu K, Liu Y, et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *Clin Chim Acta*. 2020; 505:172-175. doi: 10.1016/j.cca.2020.03.009. [Epub ahead of print]
3. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, et al. Positive RT-PCR Test Results in Patients Recovered From COVID-19. 2020 Feb 27. doi: 10.1001/jama.2020.2783. [Epub ahead of print]
4. Xiao AT, Tong YX, Zhang S. False-negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: Rather than recurrence. *J Med Virol*. 2020 Apr 9. doi: 10.1002/jmv.25855. [Epub ahead of print]
5. Yuan J, Kou S, Liang Y, Zeng J, Pan Y, Liu L. PCR Assays Turned Positive in 25 Discharged COVID-19 Patients. *Clin Infect Dis*. 2020 Apr 8. pii: ciaa398. doi: 10.1093/cid/ciaa398. [Epub ahead of print]
6. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. 2020 Feb 7. doi: 10.1001/jama.2020.1585. [Epub ahead of print]
7. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3). doi: 10.2807/1560-7917.ES.2020.25.3.2000045.
8. Tan, W.; Lu, Y.; Zhang, J.; Wang, J.; Dan, Y.; Tan, Z.; et al. Viral kinetics and antibody responses in patients with COVID-19. medRxiv 2020. [CrossRef]
9. Vashist SK. In Vitro Diagnostic Assays for COVID-19: Recent Advances and Emerging Trends. *Diagnostics (Basel)*. 2020;10(4). pii: E202. doi: 10.3390/diagnostics10040202.